The Health Impact of Chemical Exposures During the Gulf War: A Research Planning Conference

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Good morning. Without further ado, I'd like to introduce our first speaker who is Dr. Hermona Soreq. Dr. Soreq is professor of molecular biology and the elected head of the Life Sciences Institute at the Hebrew University of Jerusalem. She is also the current president of the Israeli Society for Biochemistry and Molecular Biology. Dr. Soreq studied biology in Jerusalem and received a PhD in biochemistry at the Weizmann Institute. For her post-doctoral research, she moved to the Rockefeller University in New York City, following which period she returned to the Weizmann Institute for 6 years, and moved to Jerusalem in 1986 as an associate professor. This is where she stayed except for 2 month visits for lecture series at Berkeley and the College de France.

In 1992, she won the U.S. Army Science Award of excellence and in 1996 a doctor of philosophy honoris causa in chemistry at the Stockholm University in Sweden. In Israel she is a member of the local academy's Committee for Future Technology, and her research interests include transgenic engineering, antisense applications, and the molecular mechanisms linking acute stress events and the delayed consequences in nervous system structure and function. So without further ado, I give you Dr. Soreq.

SESSION V:
Chemical Exposures: Possible Mechanisms of Action

Role of Susceptibility

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Good morning. Our aim today is to look into what possibly could have happened. A lot of the topics I'll mention today were mentioned already yesterday. One of them is the concept of post traumatic stress disorder, which is far more complex it seems than what we used to think. We in Israel write from right to left, so let's start from the right hand side here and talk about the connections between nerves or between nerve and muscle that are the focus of interest in our subject of today of exposure. We are talking about what we call a cholinergic synapse that functions by secreting a neurotransmitter acetylcholine into the cleft where it can be crossing the synapse to react with a receptor or hydrolyzed by an enzyme of acetylcholinesterase which is the target of both chemical warfare agents and a lot of drugs, insecticides and natural compounds.

We know that under psychological stress, we elevate functioning of such synapses. So that's a very short-term event. We need stress. We need to function more alertly when under stress. However, in some individuals over a long time, sometimes years, a symptom which develops which is different. It's post traumatic stress disorder. This is a magnetic resonance imaging done by Professor McEwen in New York to show that in a veteran of the Vietnam War, I think, the structure of the brain has been changed because of his condition of post traumatic stress disorder to the effect that part of the hippocampus brain has shrunk. This is the comparison to a normal patient.

So, what we're talking about begins with a very fast hippocampus excitation in the cholinergic synapse and ends with progressive long-lasting changes, even within the structure of the brain, not only it's function. And it can happen not only to those patients who were subject to an acute traumatic event at a psychological level, but also to those who were exposed to insecticides. Of course, that brings to mind the Gulf War syndrome enigma. And recently, some of the symptoms that I've talked about here are mentioned also with regard to Alzheimer's disease.

What we wanted to find out is:

‘ What are the molecular mechanisms that lead to this change in the brain structure and

function?

- Can we come up with animal models to prove our hypothesis?

- Most important, can we suggest a rescue protocol?

How did we come to be interested in this story? We've been interested in the functioning of cholinergic synapse for many years. We are approached by the Israeli Medical Corps when issues come up that are related to cholinergic neural transmission. And I was invited to be consulted with regard to the following question, which was a little bit after the Gulf War, and the question related to the way Israeli soldiers filled out prospective questionnaires after being administered with pyridostigmine during the Gulf War.

Now, pyridostigmine is shown here. It's an inhibitor of acetylcholinesterase. It is used prophylactically in anticipation of chemical warfare, and it has been shown to protect animals from chemical warfare agents, and to be relatively safe in soldiers that were treated experimentally with this drug in peace days. During the war, however, same age, same health status soldiers, young males 18 years old, filled out questionnaires, and it seemed that pyridostigmine administration was associated under the war stress with anxiety, inability to sleep, and difficulty calculating. These are central nervous system symptoms. And the concept until then was that pyridostigmine is not supposed to penetrate the brain at all because it is a charged molecule.

So, we've looked into that issue in mice. We've checked in mouse experiments whether stress conditions would facilitate penetration of pyridostigmine into the brain. You'll ask me, "How do you know when a mouse is under stress?" Well, actually, this is quite simple. What you do is you throw a mouse into a water bucket and you don't tell it that you'll be there to pull it out. That's real stress. Under that stress, pyridostigmine gets into the brain a hundred fold more effectively into the mouse brain than it does under non-stress conditions. Furthermore, even blue dyes get into the brain.

Now, our brain is protected from the periphery by a wall, that is, a physical wall and a virtual wall involving pumps and active proteins that are throwing compounds out of the brain. We called it the blood brain barrier. It's important, yet it seems to be disrupted under stress. At least it did in a mouse.

After that research, we continued to ask ourselves if this happened in humans, too. What you see here is computerized thermography of the human brain where a woman with eclampsia during pregnancy and a man with epilepsy showed penetrance of the agent, the enhancement agent that is used in computerized thermography, which we thought might be related to their mental state. We went over clinical correlates for a lot of patients while we looked at CT scans, and we could correlate with heart rates, with white blood cell counts, with cortisone levels, and with penetrance

of cerebrospinal fluid, all of which associated with mental stress.

So, let's say, well perhaps it true that pyridostigmine and maybe many other compounds get into the brain more effectively under stress. So, who would be susceptible to such penetrance? Naturally we wanted to look at the targets of cholinesterase inhibitors, and the targets are produce by 2 genes. The Human Genome committee very originally called them the ACHE gene and the BCHE gene. So, one is the key gene in the brain, and one is the one that produces the problem in the periphery. It was mentioned yesterday by Dr. Spencer as serving as the sponge that can remove cholinesterase inhibitors and prevent them from getting into the brain in overdose.

Over the past few years, we've found 2 types of mutations or polymorphisms that create intensive susceptibility to cholinesterase inhibitors. One is in the gene, in the butyryl cholinesterase gene. This is a mutation that is abundant in the Israeli population, and what you see here is a family pedigree. Where this is a soldier administered with pyridostigmine during the Gulf War, suffered insomnia, weight loss, deep depression until he got off all drugs and is much better now. This guy carries two copies of that mutation. This study was supported by the Department of Defense, and when we found that, they put together a team which checked the possibility in this country that a mutation would be responsible for at least part of the cases. However, this is a mutation that seems to be far more abundant in the Middle East than in the United States.

Next, we found that this mutation prevents penetrance of inhibitors into the protein, and this is the structure of the protein. So, we thought, "Well, we can understand that." If you don't sponge away the inhibitors of the systems, they would more often get into the brain and endanger the brain enzyme which is why those people are so susceptible.

So, next we look at all of those people that seemed to suffer acute or delayed responses to anti-cholinesterases in Israel that are referred to us. What you see here is another family pedigree. And this is a woman who took only 1 pill of pyridostigmine and got very sick for long days after. And she carries only one copy, that's why it's a half circle, of another mutation which takes place in the controlling domain of the acetyl cholinesterase gene, and that mutation surprisingly didn't cause the reduction in the protein, but elevation. So, we have in one case individuals with too little of this activator and in the other case, individuals with too much of that enzyme. And that called to look into the enigma: Could too much acetyl cholinesterase be associated with acute sensitivity to cholinesterase inhibitors.

How do you study that? We set up two models. One is the stressed mouse that I told you about, and the other one is mouse brain slices that we can expose to anti-cholinesterases in a very well controlled dose. And we looked at 2 things. First, we looked at the electrophysiology. You expose a mouse brain slice to an inhibitor and you get excitation. Now acetyl cholinesterase is the enzyme that terminates neurotransmission with acetyl choline. If you block it, you elevate the

amount of acetyl choline, so this mimics a situation of stress. So, that made sense. Then next we looked at gene expression. What you see here is that much more acetyl cholinesterase gene activity is seen either under stress or under exposure to anti-cholinesterases. So, maybe there was something to that woman's mutation.

Now, we looked at the other genes involved in the pathway for cholinergic neurotransmission. Just to make a long story short, let me show you this model. Let's think about the cholinergic synapse as a reservoir of water where the water is acetyl choline. There's an enzyme that produces acetyl choline, choline acetyl transferase. There's a transporter that packages it in the synapse, and these both are blocked. There is less synthesis under stress. Acetyl cholinesterase is the thing that empties this synapse, and this is elevated. So, we have a water reservoir where we close the tap filling it, and we open fully the tap emptying it. In other words, the brain is getting geared to down tune the stress condition. Now, we said we need stress. Let me tell you that we also need to down tune stress because we can't go on being stressed too long, or else we will deteriorate into epileptic seizures.

So, this phenomenon made perfect sense to us. The only question was: How long does it take, and what are the long-term consequences of it? So, to ask this question, we looked deeper into the expression of acetyl cholinesterase transcripts. In one gene, you can have several options for proteins. And this particular protein creates 2 types in the brain. One is getting into the synapse, and this is again the structure of the protein with its long red tail. And the other one was unknown until we found it from molecular biology. It has a shorter tail. You see immediately that it's different because it's yellow. These are the 2 proteins we needed to think about.

The third option we considered was: What happens in the brain when that protein is blocked by inhibitors? So, we mimicked that by introducing, through genetic engineering, a peptide into the active site. So, this is the same protein as in the synapse, only it cannot do anything to acetyl choline. So, we have 3 tools here. We have the 3 types of transgenes that we can introduce into an animal and ask: What happens when you have too much of this protein? And we have probes. We have a yellow probe to look for this protein, and a red probe to look for that.

So what do we need to look for? What are the events that can potentially lead from trauma, a short-term event, to post traumatic stress disorder? We would think that the trauma happens at the synapse that connects to nerve cells. A synapse in the electron microscope is just a bag of vesicles filled with acetyl cholinesterase. A trauma would change gene expression, and that would take minutes to hours. Then, during the following days or weeks, the gene products which take the place of the original proteins, and eventually, if we have a post trauma stress disorder, we would expect a permanent change in the synapse structure and function.

So we look for the production. What we find is that it's only the yellow protein that is involved. The rare lead through acetyl cholinesterase form that we found through molecular biology. This

form is over-expressed under exposure to inhibitors. The over-expression would take at least 3 days if we look in the cortex. And when we exposed a mouse for 4 consecutive days to an inhibitor, then let it rest for one month and look at the expression, this is to show you that the over-expression continues for several weeks at least after the initial event.

So, what happens when a mouse over-produces acetyl cholinesterase all its life? We created transgenic animals, introduced the genes' encoding, the active protein, the inactive protein, all the read through stress associated protein into the brain. And we looked at the properties of neurons. Now neurons have one long filament accent. This is staining from neuron filaments. And what you see here is, as compared to the normal brain, the transgenic mice with too much acetyl cholinesterase, whether active or inactive, would develop a neuropathology in axons. The stress associated protein causes much less of it. Which means that, again, nature played the game fair with us. It produces too much of this protein, but this is the less damaging isoform.

Now, apart from the axons, the neurons have dendrites. These are the thin processes that create the neuronal network. At the age of 5 weeks, the transgenic mice look normal in their dendrites, but at the age of 7 months, half a year later, they lost a lot of their dendrites, saying that the neuronal network might have been damaged. And the synapses are lost from the tips of these dendrites.

Does that affect the cognition? Transgenic mice with too much acetyl cholinesterase lose the capacity to find a platform in a hidden platform test. So, they lose the capacity to learn special navigation. They also lose the capacity to recognize another mouse which is a short-term memory test. We can fix that if we administer an inhibitor just the same as pyridostigmine or that Alzheimer's drugs that are being used to date that have been approved.

However, the next slide shows you that that treatment has a problem to it. Because we are talking about a gene that produces 2 types of proteins, and we said that these 2 proteins have 2 functions. First, they change cholinergic neurotransmission. Second, they change, and not for the better, neuronal networks. And we also see that inhibitors would induce over-production of these proteins, and that even when inhibited, and we won't see them if we look for catalytic activity, they still can cause damage.

So, can molecular biology offer a solution here? Can we say: Will there be a treatment that would block the non-wanted functions of these proteins without causing this elevation in this amount? Molecular biology teaches us today that we can use antisense technology to block expression of the gene, and the next slide shows you the concept of that. We synthesize short DNA chains that can block the RNA form producing the protein. And that would solve the problem for us. It would be as effective as an inhibitor without causing the damage. We've invested a lot of effort in synthesizing these short chains. We introduced them into the brains of mice and then, as you can see here, those mice can now recognize other mice perfectly well. We

didn't use a chemical inhibitor. We used genetic engineering here. It's a drug that can be administered in very low doses, and it solves the memory problem.

Again, English is not our mother tongue. So we go frequently to the dictionary. We looked at that and we found that stress is not only chemical and emotional stress, but physical stress is also of consequence here. And the next slide brings you an example of physical stress. This is a model of head injury that was constructed in our university by Professor Shahmad. After head injury, acetyl cholinesterase is over-expressed. We can block that over-expression by antisense administration. The transgenic mice that make too much acetyl cholinesterase make a lot of acetyl cholinesterase after injury, even in the non-inured hemisphere. But antisense agent works there as well.

The transgenics are extremely susceptible to head injury. We lose half of them under conditions that the normal mice don't die. But the antisense treatment cures them, that is, we have better survival, better recovery, and better protection of the brain neurons under the antisense treatment.

So, what did we have to date? I told you about the double-edge of an enzyme, over-expressed acetyl cholinesterase. In a resting state, we have a certain level which is just the right one for us of this protein. Under stress, it's over-produced. This can depress cholinergic activity through the catalytic role of this enzyme, but it can also cause neural anatomical pathologies for the non-catalytic activities. That could contribute to post traumatic stress disorder, to multi-organ ailments, and to many different diseases, even muscle diseases. We believe that we can, today, start asking the question: Would humans respond well to these antisense treatments that we have developed.

And the last slide. So, Israel looks very different from Atlanta. A lot of people in my life contributed to this study including Dr. Seidman who is here in the audience. And again, I would like to thank the DoD and the VA that supported this research with, I hope, future benefits to the veterans and to mankind. Thank you.

Dr. Timothy Gerrity, Moderator

Thank you Dr. Soreq for a really, extraordinarily fascinating presentation. The next speaker on this morning's agenda is Dr. Mohamed Abou-Donia who is going to be speaking on the synergistic effects of chemical combinations. Dr. Abou-Donia is a professor of pharmacology and cancer biology at Duke University Medical Center. For those of you interested, they trounced Carolina the other night. He holds a secondary appointment as a professor of neurobiology. He was the deputy director of the toxicology program at Duke University from 1981 to 1985, and is currently the deputy director of Duke University Marine Biological Center. He has been certified a Toxicologist by both the American Board of Toxicology since 1981 and the Academy of Toxicological Sciences since 1982. He has edited *Neurotoxicology*, has published more than 230

papers in peer reviewed literature. I could go on and on about Dr. Abou-Donia's distinguished career as a toxicologist. Let it just be said that he is one of the premier neurotoxicologists in the world. So, Dr. Abou-Donia.

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Synergistic Effects of Chemical Combinations

Thank you very much. During the meeting yesterday, I had been thinking about the questions that needed to be answered, and I think that we have 3 major questions that we should try to answer.

The first question that we need to answer is: Is it possible that we can produce neurological deficits or injury following low-level exposure? Dr. Spencer mentioned yesterday that there is a threshold level at which there is no effect. So, it's very important to try to answer this question whether the exposure to multiple chemicals below the threshold level would produce neurologic deficits. That is a very complicated question because that depends on the intrinsic toxicity of the chemical as well as the chemical mixture, and as we've just heard, the genetic component of the individual.

The second question that needs to be answered is the delay period. We know the veterans that were exposed to chemicals in the Gulf War developed the disease after they came here, and continued for a long time. So, we need to answer this question: What is the probability that injury would take so long to manifest itself as functional problems?

The third question that we also need to answer is the prognosis. What is the prognosis of this kind of injury? Generally, when you talk about the nervous system, the peripheral nervous system regenerates, even during exposure. However, the central nervous system damage is usually more long lasting. The nervous system, the central nervous system, the brain, the spinal cord do not regenerate. But, there could be improvement, improvement related to other neurons taking added functions of the injured neurons, and actually can compensate for that. This is an area that needs to be, again, studied.

My talk today will try to maybe answer some of these questions. Generally, individuals are exposed to many chemicals. We're exposed to medications, food additives, pesticides, fuel, industrial chemicals. We are exposed to these chemicals every day of our lives. If we look at something as simple as a food additive. If the ice cream, and we don't think about it when we eat it, has about 30 to 35 different components, ingredients. The FDA does not require

manufacturers to cite every single chemical. These are only cited by classes like artificial flavor, artificial color, fat, carbohydrate. But there are many chemicals in all of these foods that we eat, and we don't really think about it. Pesticides, as we know, are very abundant in everything that we are probably exposed to.

The interaction between, drug interactions, this has been known for many years. There are thousands of papers in the literature of interactions between drugs that are used for medications. The FDA requires pharmaceutical companies to submit data to show the safety of drugs they are going to use in combinations.

What we don't have, actually, is a regulation that would regulate and test interactions between chemicals and chemicals and drugs and chemicals. When we take drugs for medication, and we are at the same time exposed to pesticides or other chemicals, but we don't really know the extent of interaction between any drug we take no matter how safe it is and the other exposures. Houses, offices, they are sprayed with insecticides without even our knowledge. What complicates this is that individuals are not all responsive to the same chemicals as everybody else. We're different genetically and, consequently, our reactions are different.

The combined chemical exposures could be divided into 3 different categories. Combined chemical exposures could result in additivity. That means that the effect of the mixture equals the summation of the single chemicals. The synergisms results from when there is a greater than additive effect of the single components. Antagonism is that the combined effect is less than additive of single components. So, we would almost keep in mind that chemicals that may produce actually, not just additive and synergistic, but there could also be antagonistic effects.

What are the mechanisms of interaction of chemicals? There are molecular chemical interactions which result in modification of chemical properties of the chemical agent itself. This is a very common phenomenon. Following the spraying of pesticides, like parathion for instance, parathion is a sulfur containing organophosphate compound. When this sprayed, it is actually converted in the environment, in the air when exposed to an oxygen, to another form which is very toxic, 1000 times as toxic as parathion itself. So, there is interaction that could take place even before we are exposed to the chemical.

The pharmacodynamic interaction, this results from the changes in the receptor protein, or the enzyme, or as we just heard a few minutes ago, could be actually, the genetic message itself could be changed. Such interactions are very common following exposures to organophosphate. If you treat an animal with organophosphate for a long time, even at low-level exposures, the animal becomes tolerant to the insecticide. This is because of the changes, down regulation of the acetyl cholinesterase receptor. A similar effect happens in humans who are under medication, myasthenia gravis patients who take pyridostigmine bromide. Pyridostigmine is the same thing that was used in the Gulf War is actually only approved for use with myasthenia gravis.

Following long exposure to it, a person has to stop taking it because it becomes ineffective because of the down regulation of the acetyl cholinesterase receptor. So, this is the pharmacodynamic reaction.

The pharmacokinetic interactions, I'll talk more about it today, results in changes in the delivery of the chemical to the site of action. Combined exposure could result in increase or decrease of the chemical at the site of action. The site of action could be either an enzyme, or a receptor, or could indeed be the genetic message for either one of these.

This interaction could result from, the chemical delivery could result from one of these different factors. It could result from gastrointestinal absorption interactions, from protein binding, chemical metabolism, as well as excretion interactions.

If we take the first one, which is the gastrointestinal interaction, we will have to keep in mind then, most of the absorption of chemicals takes place in the small intestine because the small intestine has a very large absorption area. Its absorption surface area is about 200 times the stomach. And also, there is intestinal bacterium that seems to be more permeable than the stomach as well as the blood flow in the intestinal capillaries is much greater than that of the stomach. So, it's important for the chemicals to be delivered from the stomach to the small intestine, and the first factor is sequestration in the GI tract, then alteration in the GI motility, alteration in the GI pH, the flora as the drug metabolizes. Drug and drug interaction or chemical interaction affects these different factors.

For instance, absorption. There are chemicals with a large surface area like charcoal, chelation, or antacids, these chemicals cause absorption of drugs and chemicals. And this actually, this very phenomenon, is used in treatment of overdose poisoning, charcoal for instance, to remove the drug from the stomach. Chelation, some chemicals are produced in soluble complexes which can prevent the chemical from being absorbed like iron, iron sulfate, the iron in the pills. Antacids. These actually reduce the absorption of tetracycline by forming insoluble chelates. On the other hand, chemicals like oil, they do enhance absorption of lipid soluble chemicals like chloride hydrocarbon insecticides or organophosphate insecticides. Also magnesium hydroxide increases absorption of some drugs by forming soluble chelates.

If we look at the alteration in motility, there are chemicals, anticholinergic agents like atropine, for instance. They do decrease the motility of the stomach and they result in the decrease of absorption of the chemical. Cholinergic agents, they actually do speed absorption of drugs like acetaminophen.

Alteration in the GI pH. Many chemicals are weak acids and weak bases, or metabolize to weak acids or weak bases. And as such, they are only absorbed from the GI tract in their lipid soluble form which means that weak bases would be better absorbed from an alkaline environment, as

well as weak acids would be absorbed from an acidic environment. Even something as simple as Vitamin C would make the environment or the medium acidic. Vitamin C could increase or decrease absorption of chemicals. Just something as simple as that. Of course, Vitamin C is important, but sometimes it's not advisable to combine chemicals with each other, even as simple as vitamins.

Absorption from the skin. Typically people are familiar with DEET. DEET is insect repellent that is effectively absorbed from the skin. Actually, it has been used in the pharmaceutical industry as a transdermal enhancer to enhance the delivery of drugs through the skin. We have shown that DEET actually seems to enhance the absorption of chemicals such as pyrethrin and polychlorinated biphenyls when administered together.

Another factor that would result in the change in the pharmacokinetic of a drug is the protein binding interactions. These are serum protein binding, 4 plasma cholinesterase binding interactions. These proteins, serum proteins, and the plasma proteins have very a important function. They protect the body from chemicals such as organophosphate, or carbamates. The plasma cholinesterases act as a sponge or as a buffer to absorb these insecticides from the body and actually excrete them. Of the plasma cholinesterase, other chemicals that are of more importance are cytochrome P450 and hydrolases. Any chemical that gets into the body, it has to be metabolized to a water soluble chemical in order for it to be excreted in the kidney. The major function of these enzymes like cytochrome P450 and hydrolases is to break down these chemicals to water soluble metabolites. Most of these enzymes are localized at the liver, but the kidney, lung and GI tract have a considerable amount. There are lower concentrations in the skin, testes, placenta, and adrenals. Even the nervous system, the brain, has a very small amount of these enzymes, this cytochrome P450.

What kind of interaction would be expected from these chemicals? Some chemicals do cause induction of increased amounts of these enzymes, and some chemicals actually cause inhibition of these enzymes. Induction of cytochrome P450. Many chemicals induce P450 by increasing the synthesis of the enzyme. And this synthesis or increase is considered to be an adaptive mechanism. By the body being overwhelmed by lipid soluble chemicals, and needing to be excreted, the liver actually is induced to make more enzyme which metabolizes these chemicals and excretes them. An example of that is phenobarbital. That's a drug that is used for the treatment of convulsions, seizure. It's been shown for many years that it induces cytochrome P450 activity, and as a result of that, if we are exposed to a chemical like parathion, parathion and phenobarbital would increase the oxidation of parathion to paroxyn which is about a thousand times toxic as parathion. So, combined exposure to a chemical like a P450 inducer and other insecticides, could result in increased toxicity.

Another example is methyl isobutyl ketone or MIBK. As Dr. Spencer mentioned yesterday, that neither of these chemicals, MIBK or MEK is neurotoxic by itself. However, MIBK or MEK,

these chemicals are extremely potent inducers of cytochrome P450. So, if we are exposed to chemicals such as hexane, which is a very, very weak neurotoxicant, it is metabolized to 2,5 hexanedione that is very, very active as a neurotoxic agent. The same thing with EPN, organophosphate insecticide, is metabolized into toxin again, in the presence of MIBK.

Again, there are chemicals that inhibit P450 such as parathion itself. In the process of being oxidized, it inhibits the enzyme and then the results, it would actually decrease the metabolism and the toxification of pyrethroids and the resultant increased toxicity of pyrethroids and carbamates.

Another chemical, piperonyl butoxide, which is an inhibitor of cytochrome P450, if you go to the store and buy a can of pyrethroids, you will find that it has 10 percent piperonyl butoxide. Piperonyl butoxide doesn't have any toxicity itself, but what it does, it inhibits cytochrome P450. Consequently, it increases the effectiveness or the successful activity of this insecticide against insects. Obviously, it would increase it also in humans who might be exposed to it. So, chemical companies have known this phenomenon for many years. They have used it to the advantage of having a more effective insecticide. Unfortunately, there are no studies available on humans or animals to demonstrate the safety of these chemicals.

I'm going to talk about the esterases. Those, again, are very important enzymes. We have two types of esterases, A-esterases and B-esterases. Their function is to break down organophosphates, or carbamates, or esterase in general. These B-esterases, they do not break down the chemical. They act as a sponge to remove them from the system. These esterases also increase for other exposures such as carbamates and cocaine. Liver diseases usually decrease the activity of these enzymes because they are made in the liver. Non-hepatic diseases also decrease them. Interestingly, pregnancy results in a decrease of these enzymes which make pregnant women and the unborn fetus much more sensitive to organophosphate and carbamate exposure.

The last effect would be the secretion. Chemicals are secreted through urinary secretion through these mechanisms. So, chemicals that are, when we have multiple chemical exposure, they compete for these different pathways and actually inhibit their secretion.

The last effect is the blood brain barrier which we just heard about. The blood brain barrier is not a membrane. It's more of a concept that the cells that form the vascular system in the brain, they seemed to be tied off and surrounded with stronger cells that make it more difficult that the normal cell, but there are a series of openings that chemicals can go through. This is the blood brain barrier that you see, much more tighter cells. Again, chemicals, it has been shown that chemicals that inhibit acetyl cholinesterase produce a leak in the blood brain barrier. Heat also causes increased permeability. Stress, that Dr. Soreq just mentioned, also causes leaks in blood brain barrier in mice. We recently have shown that a combination of drugs such as pyridostigmine bromide and the pesticides like DEET and pyrethrin also increase the permeability of the blood brain barrier. Thank you very much. I'll stop here.

Dr. Timothy Gerrity, Moderator

Thank you very much Mohamed for a very elegant presentation that points out the complexity of understanding human health effects of chemicals because of the complex interactions between chemicals, and combined with the fact that we are not, in our everyday environment, exposed to single chemicals.

The next speaker on the agenda is Dr. Richard Doty who is going to be speaking to us about olfactory mechanisms and exposures to chemicals. Dr. Doty is currently the director of the Smell and Taste Center and professor of otorhinolaryngalogy and head and neck surgery at the University of Pennsylvania Medical Center in Philadelphia. Dr. Doty is an editorial consultant to over 30 scientific journals, and he has published more than 250 publications in the scientific peer reviewed literature. Dr. Doty is a member of numerous scientific associations and academies. He is best known for having developed the University of Pennsylvania's Smell Identification Test which is a 40 odorant self-administered odorant olfactory test that has been heralded as being the eye test for the nose. That should not be minimized, the importance of that, believe me. Dr. Doty received a PhD in comparative and physiological psychology from Michigan State University, and has much post-doctoral experience since receiving that degree, prior to joining the University of Pennsylvania. Dr. Doty.

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The Olfactory System: An Overview

First of all, it's a great pleasure to be here and to talk about olfaction. I think my charge today is to tell you a little bit about the olfactory system, and then maybe give you a glimpse of a few ways in which this system might relate to chemical exposure or how it is related to health.

I represent a very large organization, and many of the individuals listed on this slide here have contributed to some of the things I'll be presenting today. I won't go through all of this because of time, but to indicate that we are a team and we have a number of different individuals involved. We also have a clinic and I'll mention somewhat about patients who come to us with both problems of smelling and tasting, and some of the data I'll present today may come from that.

Now, what is the olfactory system? Basically, we have in the top of our noses a specialized membrane called the olfactory neuroepithelium. Within that membrane, we have about 6 million of what we call bipolar receptors cells. This is an example of these cells. There are cilia that

extend out into the mucus, upon which odorants bind that makes changes, electrochemical changes, in the membrane, that leads to firing off of these cells. These 6 million cells project from the nasal cavity directly into the brain without synapsing through the cribriform plate of the ethmoid bone, and then the hookup with other cells that project more centrally into the brain areas that have to do with integration of olfactory information, perception, and so on.
This is a very interesting membrane because it turns over, cells die as a result of exposure to viruses or to chemicals, and they get replaced often. Although, as we get older and for other reasons, depending upon the amount of damage, that cell replacement may or may not occur.

The glandular structure in this region, the Bowman's glands lead to secretions of mucous which protect this system. Interestingly, we were talking about cytochrome P450, actually the cytochrome P450 activity in the olfactory mucosa, or this olfactory membrane, rivals that as in the liver. So, this is an area where a lot of chemicals get detoxified. Unfortunately, many agents make their way into the brain through the olfactory route, either around theses cells or actually being actively transported directly into the brain. The olfactory pathways are a major source of viral invasion into the brain. Polio virus, many years ago in the early 30s, they used to cauterize the top of the nose with zinc sulfate in children in Toronto and other places to try to prevent polio epidemics and the polio virus reaching inside the brain. Many other things, St. Louis encephalitis virus, rabies virus, many viruses make their way directly into the brain and, in effect, bypass the blood brain barrier, if you will, through this route. There are other nerves, the trigeminal nerve, where this can occur, but the olfactory nerve is well documented.

Also, in many cases in many diseases, smell loss is present and we think that perhaps something made its way into the brain through that route. Smell loss is the first sign of Alzheimer's disease, for example. It's the first sign of Parkinson's disease. There are many interesting aspects that I don't have time to get into today. But this sensory system is uniquely positioned to, in effect, connect the outside world with the inside world, and it's nerve endings are rather directly exposed to the outside world.

This is a low power electron photomicrograph of the olfactory epithelium. Basically, the different cell types – these are supporting cells, this is a duct from a Bowman's gland. Basically those 6 million bipolar receptor cells collect in the fascicles, that is bundles, that go up through the cribriform plate. The surface area of the cilia is quite large. If you calculate it out, it's nearly 10 square inches of surface area upon which molecules bind. And these cilia have no motility. They sort of waft, if you will, in the mucous. They don't carry the mucous blanket along as the cilia do elsewhere in the respiratory tract. So, they're a different type of cilia. They are very specialized for detecting chemicals. In the respiratory epithelium, you have cilia that beat in unison, carry the mucous blanket along, and so on.

Well, the central olfactory structures that the olfactory system project are very interesting too because these are structures often that are damaged in, for example, Alzheimer's disease. If you

look where plaques and tangles arise, those are the neuropathological markers of Alzheimer's disease, they arise in brain regions that receive projections from the olfactory bulb, from the second order neurons of the olfactory bulb. So you think of limbic regions being largely involved, and the rhinal cortex, and of course the hippocampal regions and so on.

There's a lot of question as to whether agents going into the brain can catalyze events and therefore produce some of the manifestations of some of these disorder, or whether these disorders simply are very susceptible to damage. In any event, there's an interesting theory called the olfactory vector hypothesis where a number of things do make their way into the brain and maybe catalyze or initiate some of these disorders.

The bipolar receptor cells project up into the bulb and then from there, they go to various regions. Their entrance into the olfactory system is largely ipsilateral, most of the projections are of the same hemisphere, the supraform cortex, the anterior olfactory nucleus, this connects the two sides, and so on. We think of the so-called primitive areas of the brain as the major areas in which there are projections. The olfactory system, interestingly, sends projections directly to cortical regions without going through the thalamus. So, it's unique in that regard as well.

How do we measure the sense of smell? There are various techniques. I'll focus primarily on the psychophysical techniques. It's like when you go to get a hearing test and you hold the button down when you can hear the tone, and you lift if up when you don't. We can do the same things with smells. We can also measure electrophysiological changes in the brain waves using what we called evoked potential olfactometry. And in addition, we can measure autonomic responses. Although, there's another nerve inside the nose called the trigeminal nerve. When you smell something that burns inside your nose, that's actually being mediated by a different nerve. That nerve is what usually affects heart rate, respiration rate, sweating, things of this sort.

The test that was mentioned earlier is a very simple 40-item scratch-and-sniff smell test. It's now used throughout the world. Basically a patient sits down, or a subject sits down, and scrapes open a micro-encapsulated odor and then is asked to report what it smells most like, and then they mark the responses on the page. There are 4 booklets. It's a highly reliable test, test-retest reliability of this test at about .94. And, indeed, individuals follow along a continuum. Normal people typically score, depending on their age, 34 or above. Older people do more poorly. Multiple sclerosis, there's a subgroup of multiple sclerosis patients that shows smell loss. I'll get into that a little bit later. People who have no sense of smells score at about chance on this test. Individuals who are cheating the test, score below chance. It's forced choice. They have to make a response. So, we catch people that are malingering and so on.

The detection threshold testing is also done. It's fairly time consuming, but we start at low concentrations of an odorant and then raise the concentration until an individual gets it correct, and then the minute they get it correct, we can move to lower concentrations and so on. So, we

do what we call a staircasing until we get a reliable measure of their threshold at the lowest concentration that they can reliably detect.

Interestingly, if you look at the left side of the nose and the right side of the nose, you'll find that they are about equal. But if you look at the two sides of the nose together, you're more sensitive, thresholds are lower. That's a little bit of a problem because, in fact, if you take the best functioning side of the nose, your bilateral function is equivalent to that. So, it's sort of like you don't have one eye, you just test acuity and, not taking that into account, you'll have pretty good visual acuity.

We can measure event related potentials. It takes some very expensive equipment to have into an air stream a very rapidly rising stimulus, and well defined stimulus, but we can do this now with this type of equipment. In effect, we can measure the changes in potentials that are evoked by a stimulus. This is over time. This is the changes in the EEG activity in terms of the voltage. And you can see that a potential is evoked. I won't get too much into that this morning, but it is now possible to do that in olfaction.

There are also imaging techniques, and we use these now. We're very interested in, for example, areas of the brain that we previously thought were purely motor, we now find in the olfactory system to be activated by odorants such as the cerebellum. Even when the person's not sniffing, odorants activate areas of the cerebellum. It's opening up whole new vistas of how we envision central transduction.

Now, what are the factors that effect your sense of smell? Aging is a very common, important factor. This is a study of 2000 people that took that smell identification test. You can see that, after the age of 80, there's a major drop-off in ability to smell. This shows up in any test you want to look at. Women on average have a better sense of smell than men do, and they hold on to it longer. We have evidence that estrogen actually protects against damage to the olfactory system in the later years. But, these losses in later life are quite marked. Over the age of 80, 3 out of 4 people can't smell very well. Between 65 and 80, 1 out of 2, 50 percent of the population, has major difficult smelling.

Here you see the same thing with thresholds inversely plotted. You can see this with, this happens to be functional imaging, you can see that older people don't have as much activation in the temporal regions of the brain than younger people which reflects this phenomenon. Men, you'll see that there's less activation, here's some of the cerebellar activation we were talking about, than women. Smoking has an adverse effect, but it's related to dose. You can see that it's related to pack years, how much the person smokes throughout the course of their life. When you correct for age and all these factors, you still see this relationship. Interestingly, it's reversible to a large degree with smoking, although you have to wait a long time, if you've been a smoker for a number of years, to get back to normal.

Multiple sclerosis, my point here is that the areas of the brain, temporal lobe regions, that are associated with plaques, in multiple sclerosis, the number of plaques pretty much predicts what the smell function's going to be, as long as you confine your analysis to the orbital, frontal, and inferior temporal lobe regions of the brain. There's nearly a perfect relationship between how many plaques are present in those areas in a multiple sclerosis patient and their ability to smell. Inverse relationship, that is. If you look at other brain regions, there's no such association.

Interestingly, schizophrenia has been thought of as a neural developmental disorder. But we now have evidence that depending on how long an individual's had schizophrenia, their smell function varies. We don't have longitudinal data, but we have cross-sectional data that suggests that duration of illness is associated with a loss in smell function. There may be something progressive or neurodegenerative in nature going on in schizophrenia as well, at least in the limbic regions. Interestingly, what we've found in schizophrenia are much smaller olfactory bulbs and tracts, and interestingly, there is a left-right differentiation. This is the right side, this is the left. You can see this is much smaller than that. In about 24 cases we've looked at, in every case it's been smaller on the right than left. So, you can actually use MRI and the olfactory system to help in the early diagnosis of schizophrenia.

Now, in animals, and some of the things I want to get to today, how do we test an animal's sense of smell? Well, there's a lot of ways. We can do it electrophysiologically, but we can also do it behavioral and sort of get to the final endpoint. This is an example where a dog is trained to sniff 3 ports. He sticks his nose in each of these 3 ports, and one of these ports has an odor going through it, and the other 2 ports have air. We randomized that, we randomly present these things, and the dog is trained, when he detects the odor, to leave his snout in there. So, we can then vary the concentration of odor and determine, just as we can with a human, the threshold of the dog, which is several orders of magnitude lower than our own for most chemicals. And we do this with rats as well. This is just to show what we call an olfactometer. We produce different concretion of odor that goes to a rat, and here's the rat doing a similar task, in this case, the task is to sniff and to touch something, a level in effect, when he notices an odor, and not to touch it when he does not.

We can talk about sensitivity to drugs that affect olfactory activity. Catecholamine drugs such as dopamine or epinephrine, at low doses enhances olfactory sensitivity. At higher low doses, it decreases olfactory sensitivity. Drugs that affect the dopamine system, D2 system for example, depress olfactory sensitivity. Whereas the ones that affect the dopamine D1 system tend to enhance olfactory sensitivity which is then blocked by D1 and D1 receptor antagonists. So, we can have a model here for looking at sensitivity.

We talked a lot about acetyl choline. And we've done a study recently that just came out in a journal called *Physiology and Behavior*. We were looking at an agonist physostigmine, or a drug that enhances the cholinergic – oh, I'm sorry. This is a study real briefly, before I talk about that

study, on chemical hypersensitivity or multiple chemical sensitivity. This is really the only scientific study of MCS that I'm aware of looking at olfactory function. But what we found in this study was, in fact, that we didn't see differences in thresholds for an agent called phenyl ethyl alcohol which is essentially rose oil that is primarily an olfactory nerve stimulant. We didn't see it for methyl ethyl ketone as well. It has some trigeminal activity. These are thresholds. The -4.56 is $10^{-4.56}$ and the other metric there for the methyl ethyl ketone threshold is parts per million in air. You can see that there wasn't a statistically significant difference in 18 subjects that had been tested in that particular study. This is the basic study design. We looked at detection thresholds, nasal resistence, blood pressure, heart rate, respiration rate, a measure of depression, and did appropriate statistical analyses of all of those. What we found was that, people with apparent multiple chemical sensitivity actually had greater nasal resistence to begin with. Their noses, if you will, clogged up. We also found that they had a higher Beck Depression Inventory scores and a few other changes. But, for olfactory sensitivity per se, measured by thresholds, we didn't see anything there.

I'll pick up on the point with the animal work looking at this agent that increases acetylcholinergic activity. This is what we've recently shown. This is a complex task. It requires a rat, basically, to discern between 2 odorants – a 1 percent concentration, it sort of smells like bananas, ethyl acetate, versus a mixture that takes that 1 percent concentration of ethyl acetate, to which there's been added butynol. Basically, the discrimination tasks became progressively more difficult, but you can see that this physostigmine, this drug, actually enhances, this is under these 3 conditions here compared to a control, it actually enhances, where you don't get into ceiling effects with the task, it enhances the detection performance of the rats. Now, this is really early data, but it does look like a cholinergic mechanisms can influence smell function.

We know that, in the olfactory system, unlike a number of other sensory systems, that there are a tremendous amount of feedback loops into the system, so-called centrifugal fibers. And many of these are cholinergic. If you turn them off or turn them on, you can effect either the sensitivity or discrimination ability of the sensory system. Some of our research now is oriented toward understanding that because, as you know, many pesticides and other agents of this sort affect that system. And certainly, some of the exposures that purportedly occurred at the Gulf War may, in fact, have been also affecting that system. Thank you very much.

Dr. Timothy Gerrity, Moderator

Thank you Richard for a very interesting presentation. I think that this underscores what may or may not be obvious and sometimes we forget, and that is that there are a lot of natural chemical hazards in our world, poisons, etc., and that we evolved this incredible sentinal sensory organ, the nose, to basically protect ourselves against inappropriately ingesting, smelling, and otherwise coming in contact with sort of natural poisons.

Our next speaker is Dr. Claudia Miller who is going to be talking on toxicant-induced loss of tolerance and masking. Dr. Miller is an associate professor of environmental and occupational medicine in the Department of Family Practice of the University of Texas Health Sciences Center at San Antonio. She is board certified in allergy and immunology and internal medicine, and holds a master's degree in environmental health. Currently, she conducts research on the health effects of low-level chemical exposures. Dr. Miller has had numerous appointments on federal advisory committees including the Department of Veterans Affairs Expert Panel on Gulf War Veterans Illnesses, and she is co-author of a WHO award-winning New Jersey report on chemical sensitivity, and a professionally acclaimed book, *Chemical Exposures: Low Levels and High Stakes.* Dr. Miller.

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Toxicant-Induced Loss of Tolerance and Masking

First, I'd like to acknowledge the VA, especially in Houston, for allowing me to come down and interview so many Gulf War veterans and spend 4, 5, 6 hours individually with them to try to get an understanding of this illness. I think that's much to their credit. Many of the patients, I think some 80 or so that I've seen including Jason Whitcomb, I want to thank them and acknowledge their help in the value that one can derive from spending time learning from your patients. There's a saying in medicine, "Listen to the patient, he's telling you the diagnosis." And if we listen long enough, maybe we'll learn.

I'd like to talk a little bit about a concept that is fairly new and really describes a number of observations that have been made in different countries. Literally, in about 13 countries now, there are reports of people developing health problems following well identified chemical exposures – everything from sick buildings, to solvent exposures, to pesticide exposures, to Gulf War veterans – and people saying that they have this initial event, initial exposure to chemically diverse substances. Subsequently, a subset of individuals in those exposure situations report losing tolerance, or experience this, and become more sensitive to very low level exposures to common everyday items like foods, medications, alcoholic beverages, caffeine, and many common chemical inhalants. Subsequently, their symptoms are triggered by those exposures. So, there are 2 steps here. We're talking about a 2-step mechanism which looks a little bit like allergy, but in fact, we do not believe that involves allergic processes.

Some of the things that you've heard this morning on this panel maybe very relevant in terms of chemicals inducing changes in neural networks, in messenger RNA systems, in some of the

olfactory and limbic areas. We don't know the specific mechanisms, but we have this phenomenonology. This triggering then occurs. So, you've got lots of tolerance initially from an exposure event subsequently triggering by common low level exposures, resulting in symptoms. Now, what this mechanism does is take into account reports in many countries, and it reflects the observations that people will have continuing problems well after the initial exposure event. One of the problems with understanding the Gulf War veterans' illnesses is, why would people still be sick 3, 4 ,10 years later. Yet, many of those exposures have ceased. This is a mechanism that would offer some understanding.

So, when veterans came home from the war, they may have run into exposures now, common everyday problems with indoor air pollutants, tobacco smoke, beverages they used to drink now making them feel ill, alcohol, caffeine. And the symptoms they have may be things like fatigue. And here comes the physician in our Titanic running into this iceberg, and we make a diagnosis based upon the symptoms alone. So, if it's fatigue and it looks like chronic fatigue syndrome, it may get that designation. It may look like a migraine headache and get the diagnosis of a migraine. It may look like depression. It may look like asthma. As we diagnose these things based upon clinical symptoms and signs, and may not always know what went on before the onset of this. We certainly know asthma can arise from many different kinds of exposures whether it's a pollen, a dust, a mold, certain chemicals like toluene, diazocyanide. But, here's a different mechanism we're talking about that may lead to symptoms, and then diagnoses which are really only labels. When physicians give these diagnoses, we often think that they've solved the problem, they've found a cause. But depression is really a descriptive diagnosis. It doesn't mean it's the origin of the illness. It could have other causes. And what we're saying here is perhaps there are chemical causes for some of these things.

Now, masking is what hides people's awareness that their symptoms may be related to exposures. And what is masking? Well, not everyone is aware that they have trouble with chemicals and it may be because they are sensitive or intolerant of common exposures. So, they might get up in the morning, use some hair spray, work around their gas stove, be exposed to traffic exhaust on their way to work, get to work – maybe in a sick building with air contaminants, be around people's fragrances, tobacco smoke, and all during the day, one exposure after another, to the point where they feel sick all of the time. At any particular point in time, they are unable to say one of those exposures is making them sick because they can't see the signal through the background noise. There's too much background noise. They just feel like they have chronic fatigue, chronic flu, and they can't sort it out. Occasionally things will become apparent. Some days they will break through that and they'll recognize something, and we'll talk about why that occurs.

So, this is the concept, toxic-induced loss of tolerance. And the question has been raised, could this be responsible for things like multiple chemical sensitivity, attention deficit disorder, depression, migraines, seizures, cardiac arrhythmias, asthma, reactive airways dysfunction

syndrome, irritable bowl, fibromyalgia, various rashes, chronic fatigue syndrome, some of the implant syndromes, Gulf War syndrome? – A whole host of things that I think you'll recognize. We don't have great knowledge always of what the etiologies are. What are the causes underlying them? So, this is a theory that asks this question.

Now, what is the evidence that this might exist? Similar reports of illnesses in different regions, in countries, in very different demographic groups. For example, in New Zealand, we have radiographic technicians who work around some of the x-ray developing chemicals used in the new high speed processors. And they're reporting having something they call darkroom disease. Darkroom disease looks all the world like chemical sensitivity and Gulf War veterans' illnesses, multi-system symptoms, multiple intolerances. I've been invited over there to speak, so I've had first hand contact with many of these people. Another group that we're seeing multiple intolerance in is the sheep dippers in the United Kingdom. Multi-system symptoms. They use organophosphate pesticides to treat the sheep. Another group in Germany, householders who have built homes out of logs preserved with pentachlorophenol. The pentachlorophenol out gases, but it out gases into the house too, and they're breathing pentachlorophenol continuously when they're in the house. And they also report multiple symptoms and intolerances. In Germany, it's called wood preservative syndrome.

In Canada a group of hospital workers, where they took an anti-corrosion agent that was added to the boiler water and used that same water for humidification in the hospital, and many hospital workers, doctors, nurses who are now chemically intolerant, chemically sensitive as they say. In the Environmental Protection Agency in Washington, my favorite example, when they installed new carpeting in a building that was poorly ventilated and had low levels of hundreds of volatile organic chemicals present. Poor ventilation in many areas, and people developed sensitivities, about 30 of the individuals now have multiple chemical sensitivity, including individuals who are attorneys and people with master's and PhD degrees actually in environmental health. Then you've got casino workers that Jim Cone talked about the other day, who were exposed to pesticides in different combinations, who couldn't count their cards anymore and became confused. And the OSHA inspector who came in had trouble thinking, too. Several of those, about 15 to 17, have gone on to report multiple chemical sensitivities. Not everyone who is exposed in these episodes goes on to develop these problems. But a subset do, and we don't understand what all the predetermining factors are. Probably it's very complicated.

But, you can see, we have reports now from over a dozen countries. It goes back historically. If you look back around the 1960s in California, they were identifying agricultural workers. About 20 percent of those who had had a prior acute exposure to organophosphates said they could no longer tolerate even a whiff of pesticides, and most of them got out of the business of working as farm workers. Another group that was reported on, actually also in the 1960s, Sveigelburg in Germany looked at chemical munitions workers, people that produced chemical weapons in Germany during the war, and 15 years later they had multiple symptoms, fatigue and the things

that we're talking about here today, as well as reporting intolerances to alcohol, nicotine, and various medications.

So, there's a theme here, and that's what we're talking about with this toxicant, toxic exposure, inducing loss of tolerance. Now, complaints of intolerances are not just for chemicals, and I think this is one of the more compelling pieces of evidence to a researcher. We're not talking about people that say they suddenly dislike being around the chemicals they work with, and don't want to work. Rather, we're talking about people who used to enjoy eating particular foods, you know, eating chocolate and having some pizza and beer, and things that now they say they don't tolerate. Many of the Gulf War veterans I have seen say one drink now of beer will either make them feel inebriated, but more often, severe withdrawal, hangover symptoms. Medications, taking a single decongestant. No problem before the war, and now some of them say they'll feel strung out for several days if they take a certain medication, a decongestant in this case.

This illness resembles addiction. You know, I talked about caffeine and alcohol, and I'll talk about nicotine. There's a resemblance here to addiction, but rather than being addiction, what we think we're dealing with is rather the flip side of addiction. And we'll get into that a little bit more. There's a plausible anatomic locus, certainly the central nervous system and the brain, and then there's some recent animal models. You heard about one of them earlier from Dr. Sorg. Now, we've looked at numbers of individuals who report being chemically intolerant. Those exposed to an organophosphate pesticide. In the purple here are people exposed to a building where remodeling went on, like the EPA building. And what's striking is the ordering of their symptoms in different organ systems and the Gulf War veterans, and this is the first 59 who came through the regional referral center in Houston, is very similar. In fact, it's statistically similar. Muscle related symptoms, head related symptoms, difficulties with concentration and memory, mood changes, heart symptoms and so on. You'll see a questionnaire, which I believe has been handed out to you, and it will reflect these different scales that are on here, so I don't want to spend time on that.

Now, among the Gulf War veterans that we saw in Houston, of the first consecutive 59, 78 percent reported new intolerances for a variety of chemical exposures, and this included mechanics who used to enjoy being around diesel exhaust. One of the fellows told me that his idea before the war of the perfect perfume was WD-40. And now he says being around that makes him very ill as does his wife's fragrances, his own fragrances that he used to enjoy, and many other common chemicals, pesticides, cleaning agents, and so on. Judging by the response we had yesterday, I am assuming that many of the veterans in this room have already experienced some of these problems as well. Medications, about 40 percent of those who had taken medication had had adverse reactions to them, and these are oftentimes things you find in the *PDR*, but you see them more often in these people. Things like problems with the decongestants, antidepressants, getting more of the side effects with antidepressants. In fact, one individual developed a tardive dyskinesia-like syndrome. Alcoholic beverages, about 2/3rds of those who

used alcohol said that now, one drink of beer, in the case of the multiple chemical sensitivity patients who are mostly women actually in this country, they'll say red wine or white wine, but the Gulf veterans it's beer. And they'll say one drink of that will make them feel ill, and many of them have given up using alcohol. Caffeine. Only 1/4th of those who used caffeine recognized caffeine intolerance, and yet many of the veterans were drinking not 1 or 2, but maybe 4, 5, 10, up to 15 or 20 cups of coffee or tea a day. Now why were they doing this? Well, they were so tired that they found they could get at least a little lift temporarily from the caffeine, so they were taking it more and more often.

Now, remember I said this phenomenon looked a little bit like addiction. What we see is people, for example Gulf War vets, after these kinds of exposure events, reporting problems with becoming addicted to something like caffeine, at the same time moving away from being what I call abducted, moving away from common chemical exposures and maybe alcoholic beverages. So, people can respond either way. They may addict or abdict. This is the result of, potentially, this loss of tolerance they've experienced. They're having more pronounced responses, stimulatory withdrawal responses, just more effect from the same amount of drug than they experienced before the war. One fellow told me, let's get down here to the tobacco, about 3/4ths of the individuals who use tobacco said that smoking one more than their usual cigarette or using someone else's stronger brand would make them feel ill, dizzy, lightheaded, nauseated. And one veteran told me that before the war, he had quit smoking, no problem, cold turkey, he got to the Gulf and started smoking again. This time, when he got home, after he'd returned, he tried to quit and he went through terrible withdrawal. He couldn't quit. It was like he was addicted and couldn't quit. So, again, this is a loss of tolerance manifesting as an addiction that he can't break. And we suspect he's very sensitive to it, but by getting another hit and another hit, he maintains his level of comfort, but if he stops, he will go through withdrawal. So, this is the concept we're talking about here. Now, think about what we know about addiction and the mechanisms. Not very much. There's a lot more that we need to know.

But, in effect, we think this may be a flip side of addiction that we're talking about, addiction and abdiction. The other important point back here about caffeine is that caffeine is something that, if we thought caffeine was causing your headaches, the way we would test you is not by giving you another cup of coffee and asking you if you get a headache. What would we do? We would stop all caffeine for a while, usually about a week. I'm an allergist, we do this all the time. Stop it for a week. If you go through withdrawal, that tells us that you may be sensitive to caffeine. If we want to be really careful and scientific, we might give you then a pill with either caffeine or no caffeine to see if your symptoms come back a week after we've gotten you withdrawn from the caffeine. This is how you test for these intolerances, directly and individually.

And then finally, foods. Individuals with this problem report food intolerances, either specific foods bothering them and/or feeling ill after meals. And the way we would explore this illness after meals is by putting people on an elimination diet, testing one food per meal to find out what

might be triggering symptoms. Note that the things we're talking about here are all chemically dissimilar. When you deal with addiction, you deal with things that are chemically dissimilar. You have only to go to Las Vegas if you have any doubt about that and you see people smoking, using alcohol, and lots of caffeine. So, there are addictions that go together and there are cross-addictions, just as we think there may be cross-abdictions going on, avoidances that are across different chemical lines.

This is a summary slide of the first 59 veterans. Thirty-six of them had intolerances they reported to chemical inhalants, foods and medications, alcohol, nicotine, or caffeine. So, the majority had reports of intolerances in all 3 categories, and there are only a small subset that had no intolerances at all. Now, when you go to see a physician, you will report to them, if they're a neurologist, your headaches. If they're in endocrinology, you'll talk about those kinds of symptoms. But, if you go see a doctor, it's unlikely you're going to talk to them about the problems you're having tolerating alcohol. You know, the response you're likely to get is, "Well, that's good. You know, don't drink it." And yet, this may be one of the hallmark symptoms, these chemical intolerances, food intolerances, caffeine and alcohol intolerances, of this illness. Just like with infectious diseases. Whenever we see fevers, we think about working up an infection. It's a hallmark symptom. Here, we're talking about chemical sensitivity and other intolerances as a hallmark symptom for this class of diseases, for this toxicant-induced loss of tolerance.

There are animal models that have been discussed and that have many of the features of toxicant-induced loss of tolerance. The time dependence sensitization that Barbara Sorg talked about yesterday. Another very interesting one that fits very well with this panel is called cholinergic super sensitivity. Dr. Overstreet has looked at animals exposed to diasopropylfluorophosphate, an organophosphate, and bred a line of rats that were especially sensitive to that organophosphate. They would drop their body temperature by several degrees. He found that these rats were not only sensitive to organophosphates, but also to ethanol, nicotine, dopamine agonists and antagonists, 5-hydroxytryptophan agonists, benzodiazepines. And then he sent some to Canada to test them for gut permeability, think about the food intolerances, and we think that's a basis for food intolerance. And in fact, they had increased gut permeability when you tested them with ovalbumen. So, here's a cholinergic rat, if you will, that mimics many of the things we're seeing in humans.

Addiction and abdiction, certainly add means toward, moving toward, abdiction, away from. This just means to advocate or proclaim. So, we're talking about addiction and abdiction occurring in the same individuals. With addiction, what we know is that when people are getting the next hit of caffeine or the next hit of nicotine, they're trying to avoid going through withdrawal, maintaining the upstroke. In the case of people who avoid a substance, it's because they recognize that the withdrawal symptoms are so unpleasant, or the stimulatory symptoms are so unpleasant, maybe they get a panic attack or very hyper or anxious, that they avoid, they move

away from the substance. So, addiction and abdictions, again, are flip sides of the same issue.

Now, what are some of the comparisons we can make between the two? Both are reported in the relevant populations to lead to the various stimulatory and withdrawal symptoms, symptoms with onset and offset of exposure. You have multi-system symptoms occurring in both cases. Think of this as chemical sensitivity. Think of this as addiction. Central nervous system symptoms predominate. You have cravings and binging reported. In the case of the chemically sensitive patient, frequently it's to caffeine and various food. Habituation occurs with repeated use, like if you continue to use various things like caffeine, then you will habituate to it. Enhanced sensitivity following a period of avoidance. In the case of tobacco we know that's true. Genetic predisposition. Gender predominance, and so on. I'm not going to read through all of these because they are in the handout that you have. But the comparisons are quite extensive here, and I think it bears looking at.

Patients in both cases are viewed as very difficult and demanding. When you have an illness that doctors don't know how to diagnose, and they're giving you medications, and you say you get worse on them instead of better, doctors don't like that. And they can be very frustrated, too. And the comparisons go on here.

The way to address this, people have said, is to actually put people through a detox kind of situation. Remove them from common exposures that may be triggering symptoms – chemicals, foods – just a very clean hospital ward where you minimize chemical exposures and see if their symptoms improve, and then go back and do blinded challenges to see if their symptoms reoccur. If you don't blind it, you could also use it actually for diagnosis and treatment.

So, this has research value, treatment value, and diagnostic value. I should say that this has been a proposal in the area of chemical sensitivity for the past 10 years. Because of costs of undertaking such a study, it has not been done yet by the federal agencies that are very interested in this approach. I have more I could say, but time is running short, so thank you very much.

Dr. Timothy Gerrity, Moderator

Thank you Dr. Miller for an interesting presentation. We're running almost 20 minutes behind schedule. We would, however, like to have a full time for the panel discussion and I'm just looking at what our, we normally would have been going for a break at ten o'clock. If we're running 20 minutes late, if we go the full session with panel discussion, that would end up being at 10:20. If we tried to keep the break down to 10 minutes, then we would begin the concurrent work group panels just about 15 minutes late, and I think that would be fine.

Drue Barrett, PhD
Chair, Conference Executive Planning Committee

Chief, Veterans' Health Activity Working Group
Division of Environmental Hazards and Health Effects
National Center for Environmental Health
Centers for Disease Control and Prevention
Atlanta, Georgia

Tim, if you could try to stop at 10:00, because we need to split up the rooms to get ready for the workgroups. You'll have 20 minutes for a discussion.

Dr. Timothy Gerrity

Okay. Then let's go.

Discussion

Kathleen Hannon, MD
Orlando, Florida

In my experience, chronic fatigue is epidemic in women. In fact, all my nannies have to have chronic fatigue, and it's just getting increasingly greater in women. In Orlando, we have a high Latin population, and for Latin women, they need to wear high heels and apply lipstick 20 times a day. Well, they can't wear high heels, and that's because they have peripheral neuropathy. And they apply that lipstick. So, what's in that lipstick? When you apply the lipstick, you may have cracked lips or you may get some lipstick on your teeth and you may absorb it through the mucous membranes. Would you like to know what chemical's in the lipstick? Squalene. And what else is squalene in? Squalene is in our body. It's in cholesterol, it's in the oils. And where is cholesterol made? In the liver. Why are people having liver transplants? This is an autoimmune disease against squalene, and people are getting sensitized by applying lipstick that has squalene in it, and possibly other oils in the lipstick. And that's why women have more of a preponderance of chronic fatigue. Thank you.

Dr. Timothy Gerrity

I think it is very important to bring up a number of etiologies, and there are the work groups on etiology at which you will have opportunities to present your ideas on etiology. I'd like us to focus on the presentations given here and have discussion that centers around these presentations, and for you to have some specific questions about those. Thank you. Next.

Leslie Simpson, PhD
Red Blood Cell Research Trust

Dunedin, New Zealand

My interest is in the significance of the maintenance of blood flow for normal function. Professor Abou-Donia referred to blood brain barrier dysfunction. Now the normal capillary is simply a tube of basement membrane which probably is a [inaudible] gel and therefore exhibits pressure dependent depermeability. But the endothelial cells in the blood brain barrier, they have tight junctions, so that's the first barrier to passage out of the capillaries in the brain. It seems that the first stimulus for this to occur must be some action of whatever drug is being examined on the endothelial cell. That's the first point. But the second thing is, if you're going to enhance transpiration, then there must be an increase in intra capillary pressure. This implies that there is a change in the blood flow properties themselves. Probably the agent is causing a stiffening of the red blood cell. The Evans blue change in the brain which Professor Soreq showed is probably evidence of the same type of change, and I would think that the weight of that brain would also be increased? Was that so?

Dr. Hermona Soreq

Well, thanks for the question which was actually raised by others too. That is exactly why we're doing the retrospective study in humans. We find no correlation whatsoever between blood pressure changes and the penetrance of drugs to the brain.

Dr. Leslie Simpson

I'm not talking about blood pressure. In the capillary, the determinant of flow is the stiffness of the red blood cell.

Dr. Hermona Soreq

Yes. But that was not checked.

Dr. Leslie Simpson

It does not necessarily produce a change in the total blood pressure.

Dr. Hermona Soreq

You may be very well right.

Dr. Leslie Simpson

Professor Doty showed changes in olfactory sensitivity with increasing age. After age 60, there is

an increase in the stiffness of the red blood cell as shown by filterability. Plasma viscosity increases, total blood viscosity increases. So, what your draft showed very nicely, was the type blood changes which occur in the elderly, implying that olfactory sensation is also a factor determined by flow to the organ. In the reduction in flow, as shown in schizophrenics, they too have blood flow changes. The packs per day associated with smoking, there is a big literature which shows that the smoker has an increasing rate of stiffening of the red blood cell . . .

Dr. Timothy Gerrity

Excuse me sir. Could you get to your question? The reason why I'm saying this is you have 6 people behind you. We've already consumed 5 minutes of 20 minutes, and I think in fairness, the other folks need to be able to ask questions.

Albert Donnay, MHS
Director
MCS Referral & Resources
Baltimore, Maryland

Thank you all for your interesting presentations. My question is for Dr. Doty, but I welcome comments from any of the panelists. I'm curious to know, you talked more about the olfactory nerve, but there are people with multiple chemical sensitivity who claim to have no olfactory sensation. They are completely anosmic, and therefore that would suggest some other pathway. One other possibility would certainly be trigeminal because these people do react to irritants and claim that those bother them. I have learned that the olfactory pathway is mediated by carbon monoxide as a neurotransmitter. I wonder if you could tell us what the neurotransmitter is in the trigeminal pathway, and if we know it yet, in the vomeronasal pathway, cranial nerve zero, and whether or not these neurotransmitters, how they play a role in habituation. When we enter a new environment, we receive novel stimulus, olfactory stimuli. If we're healthy, we can habituate and zone out those odors very quickly? Hydrogen sulfide's a good example. How does that happen?

Dr. Richard Doty

Well, let me address the first part of your question addressing the anosmia in multiple chemical hypersensitivity. If you look at least in the traditional sort of classic descriptions of that disorder, or family of disorders, anosmia has never been a component of that. It's always been either hypersensitivity. Certainly none of the people that we've seen that we've looked at that met criteria that we set up for that disorder were anosmic. Certainly, people can have allergies, and can have, and I'm not claiming the olfactory system by the way is the root or the source of these sensitivities. I just was describing the olfactory system in a more general sense of how it does allow things into the brain, however. So, I've never, I don't know anything about anosmic

chemically hypersensitive people. They may be out there and that would suggest that maybe that's not the major root of something. But on the other hand, you could argue against that, so I don't know quite how to relate that.

In terms of neurotransmitters, some of the amino acid transmitters are involved in the olfactory system. But you've got to remember, it's a very complicated system. There are dozens and dozens of neurotransmitters involved depending on which cells you're looking at, and which areas you're talking about. In terms of the vomeronasal organ, I don't know to what extent the neurotransmitters involved have been isolated. Things are changing daily in this field. That's an area that I haven't read in a while, so I can't really bring you up to date on that.

Mr. Albert Donnay

Trigeminal was the other one I asked about.

Dr. Richard Doty

Trigeminal. I'm not sure there either about the underlying, there's probably people more knowledgeable about that. But, there are a number of different answers depending where in the system you're looking. I really can't answer that.

Satu Somani, PhD
Professor of Pharmacology and Toxicology
Department of Pharmacology
Southern Illinois University School of Medicine
Springfield, Illinois

This question is for Dr. Soreq. Dr. Soreq, you explained very well about the blood brain barrier under stress conditions, and the slide which you showed at the end indicated that under stress, the blood brain barrier opens. Is this the same for the closing of the blood brain barrier. Is this the same phenomenon that occurs as you explained for the opening of the blood brain barrier?

Dr. Hermona Soreq

That's a very good question. From our studies and from studies of others, it seems that the opening of the blood brain barrier under stress is a transient phenomenon that takes at least 45 minutes. However, it's a very complex process just as Dr. Abou-Donia was saying. This is relevant to both junctions and proteins, like the multi-drug response protein, to fluxes of compounds through these cell walls, in two directions – inside and outside. While we are sure that both the blood brain barrier itself, and most probably it's opening under stress, have physiological functions. We do not know which are the molecules that control the opening yet,

and again, this is one of the main directions of our current research.

Dr. Satu Somani

A continuation of that, are the free radicals involved, because . . .

Dr. Timothy Gerrity

Dr. Somani, I'm sorry, there are just so many people that want to ask questions. I have to limit you to one.

Dr. Anne Solomon
Research Fellow
Department of Medicine
Pennsylvania State College of Medicine
Hershey, Pennsylvania

My question is directed to Dr. Soreq. One of our colleagues in Baltimore, Gary Rockwood, has been trying to replicate your studies, and I spoke with him before I came and have brought the papers with me. There have been some difficulties, I understand, in replicating some of the studies. Not in your lab, I know. But it seems that the transgenic mice may be the element that is making the difference. I thought if you'd like to perhaps comment on that in terms of expanding your topic of susceptibility.

Dr. Hermona Soreq

Absolutely. Thank you for the question. I need to emphasize here that the experiments I reported on were not done in transgenic mice. These were in perfectly normal mice. I went through a lengthy correspondence with Dr. Rockwell, who developed a totally different protocol, and he wrote me that that protocol doesn't work, and I repeated in very great detail what we have done. But he elects to use his protocol, which is definitely his right, and that protocol doesn't work.

Dr. Anne Solomon

Okay, fine. Thank you. Fair enough.

Beatrice Golomb
Health Consultant
RAND Corporation
Santa Monica, California

A comment on a related question to add to the comments by Drs. Abou-Donia and Soreq on blood brain barriers permeability. There are several other potential sources that might lead to permeability. One is adrenergic agents, and another interesting one is that headache itself induces a neurogenic inflammation. And, in fact, the reason the migraine drug sumatriptan is effective only in moderate or severe headache is because only in that circumstance is the blood brain barrier permeable enough for it to enter. The related question, since I'm interested in pyridostigmine bromide and how it might enter the brain and have effects, is for Dr. Soreq. As you know, I'm interested in the issue of whether down-regulation of acetylcholine could be related to symptoms in Gulf War veterans. There are many elements that are compatible. So, I find your research very interesting. But, there are other potential down-regulatory mechanisms that have been shown with acetylcholinesterase inhibiting agents, including reduced release of acetylcholine, withdrawal of nerve terminals, reduced receptor density, at least for muscarinic receptors, reduced receptor sensitivity, and reduced receptor affinity. I was wondering if you're looking at any of those others or the time courses of those effects?

Dr. Hermona Soreq

Yes. Thank you. We are doing experiments on at least part of the very long list you suggested. So far, none of the changes we could find were as dramatic and rapid as the ones that I reported on.

Mary Lamielle
Executive Director
National Center for Environmental Health Strategies, Inc.
Voorhees, New Jersey

My question is directed to Dr. Miller. If what you say is true, what do we do about the issue of case definition?

Dr. Claudia Miller

This has been a thorny question just as it has been with the Gulf veterans' illnesses and chronic fatigue syndrome. What do you do with this chemical sensitivity issue problem in terms of a case definition? If what we're dealing with here is a class of diseases, with different chemical exposures initiating them, and now different multi-system symptoms coming out as a consequence, we may be dealing with something that's almost parallel to dealing with different infectious agents leading to different kinds of clinical conditions. When you're dealing with a class of illnesses, trying to apply a case definition to this TILT class of illness is like trying perhaps to apply a case definition to all infectious diseases – not an easy thing to do. You can take a particular infectious disease and say, "All right, you've got a certain kind of rash with this sort of presentation – it's lyme disease." But, it's very difficult if you're dealing with all infectious

disease. And I think we may be dealing with a whole range of chemical exposures (solvents, pesticides, combustion products, and so on) which can interact with people and lead to multi-system kinds of symptoms. So, how do you come up with a tight case definition, which medically we'd like to have very specific symptoms, signs, something you can hang your hat on and say, "Yeah, this is what's going on." In California, a meeting sponsored by the Superfund agency tried to explore this question of case definition, and the consensus was that it may be better to try to evaluate people along the dimensions related to their chemical intolerance. For example, a scale on their symptoms and the symptom severity, a scale on chemical intolerances and how severe those are, a scale on other intolerances like alcohol, caffeine, nicotine, a scale on life impact of these exposures provided people aren't too masked, and finally we've added the idea of having a masking scale which really tells you (and that's on that questionnaire that you all have) tells you what might be ongoing exposures an individual may have that could interfere with their recognition that they are having chemical intolerances. So, if they're very masked, you know, smoking and around alcohol and caffeine, fragrances, and everything else, they may not be aware that they are sensitive to some of those things and various chemical exposures. So, it's very important, this idea of unmasking people, removing them from the background of exposures temporarily, and testing to see what might be causing symptoms, and then looking for, okay, what's the mechanism involved here?

Related to this, in the area of drugs, we know that cocaine, for example, is called a very dirty drug because it hits so many different receptors. And it's very hard to find a drug, in fact, no one's found a drug to treat cocaine addiction per se. So, this may be a similar kind of phenomenon. Many different chemical exposures triggering symptoms in different organ systems. And you may have opiate systems, you may have cholinergic systems, you may have serotinergic systems being affected by these processes. And finding one drug that's going to handle all that may be extraordinarily difficult.

William Meggs, MD, PhD
Associate Professor of Emergency Medicine
Vice Chair of Clinical Affairs
Chief, Division of Toxicology
Department of Emergency Medicine
East Carolina University School of Medicine
Greenville, North Carolina

Question for Dr. Soreq. First, I want to thank you for putting some solid science behind something that we clinicians have observed for many years, namely that there are people exquisitely sensitive to organophosphates and there are also people who, after an organophosphate poisoning never get well, they never get their brains back. These substances are used in homes, they're sprayed into the air of homes, schools, and offices in this country. My question is, based on your work and what you know about them, should this practice be banned?

Dr. Hermona Soreq

Well, this is a loaded question. Organophosphates are used throughout the world, and mainly in agricultural countries like ours, too. It's a problem. They increase the yield of agricultural crops to an extent that mankind cannot afford to stop using them until we've got a better substitute. I would definitely advocate much more carefulness, much more caution in the use of these compounds. I see people here with masks. No one really takes care of using those masks when they spray their homes with these compounds. Personally, I'm much more careful after we saw those findings. I think that that would be a good rule for all of us. In our country, for example, there is a law that prohibits the use of spraying, I think 3 weeks before you're commercialize agricultural products. But, there is no mechanism to enforce that law. Maybe this is something we could wish for – better law enforcement in the carefulness of using these dangerous compounds.

Dr. Timothy Gerrity, Moderator

Okay, I'm going to have to cut off questions right now because . . .

Dr. Drue Barrett

You can take one more question.

Audience Member

Thank you. It's about the nose and smell. This is for Dr. Doty. Is odor, in and of itself, a de novo agent or factor for triggering some problems? As you had said, it goes straight to the brain, it's one of the unique features, and then starting a series of problems like triggering the MCI-like symptoms.

Dr. Richard Doty

That's a very good question. It's sort of like if you say, "Does bright light cause certain symptoms?" And it probably does in some individuals. So, I suppose that, at least theoretically, a certain odor might do that. Although, usually, it's the other way around. There may be conditioning that occurs so that odors take on a life of their own. If they've been conditioned with illness, then somebody experiences that odor, and then they get ill. So, there can be a lot of other factors than a strict hard wired kind of notion that conditioning can occur in a particular odor that's salient when people get sick, we know very clearly, both with taste and odor aversion conditioning, that that can happen. So, it's hard to dis-entwine the effects, and the conditioned effects, and so on. But certainly it's a theoretical possibility perhaps in certain cases.

Dr. Claudia Miller

May I comment?

Audience Member

You mentioned conditioning effect, may I just quickly mention, for the sake of preventive medicine and public health, that I read recently, for example, Oxford Press in the U.K. is producing for children books that they will scratch and they can smell, and they are producing some more. They are already stimulating the public to watch for this. The first edition is the *Stinking Royals* where William the Conqueror supposedly died because he fell from his horse, perforated his bowels, and when they were entombing him, the bowels burst and produced this stinking smell. And a child is supposed to rub this and scratch this. And they're coming out with *Horrors for Halloween*. Is there a potential impact for this conditioning and the olfactory system damage?

Dr. Claudia Miller

You know, you're raising a question about olfactory sensitization in this kind of odor conditioning. It's a very important question. Remember that first of all, there are anosmic patients, and some of us have seem them, who are saying they are chemically sensitive people, who get problems with ingestants and with skin contactants, and suppositories, and IVs of various drugs. But beyond that, if you want to test this question of odor conditioning versus response to the chemical, do blinded placebo control challenges under the appropriately controlled conditions to resolve that very important question, because I think it's one that's key in this area in terms of people's acceptance of whether this is or is not psychogenic. And it may be different in different people.

Audience Member

Because we are here talking about low-level chemical exposures.

Dr. Timothy Gerrity, Moderator

Thank you. We're going to have to close off this session. But before you go on your break, Phil Talboy with the CDC has something to say.

Mr. Phillip Talboy
Co-Chair, Conference Executive Planning Committee
Deputy Chief, Veterans' Health Activity Working Group
Division of Environmental Hazards and Health Effects

National Center for Environmental Health
Centers for Disease Control and Prevention
Atlanta, Georgia

I just wanted to say moving into session 2 which is what we have designed for the public input session, the purpose of the conference is to provide research recommendations to the scientists. We have designed session 2 of the work groups for the public to provide their input. What we ask is that during that input session, that we focus on research recommendations due to the fact that our time is very, very limited. We've asked the chairs to utilize the timers, as well as enforce the amount of time that we have for each person to speak because we have many names on the list and we want to give everybody the opportunity to provide input. The amount of time each will depend on each different work group themselves, how many people are signed up. The chairs will let you know prior to the start of it. Thank you.

The session adjourned.

SESSION VII:
Studying the Health Impact of Chemical Exposures During the Gulf War: Methodological Considerations

Stephen Thacker, MD, MSc, Moderator
Director, Epidemiology Program Office
Centers for Disease Control and Prevention
Atlanta, Georgia

Thank you. Welcome to the session *Studying the Health Impact of Chemical Exposures During the Gulf War: Methodologic Considerations.* So, we have a bunch of methodologists here. I'm Stephen Thacker. I'm the director of epidemiology at the Centers for Disease and Control and Prevention. I'll serve as the moderator. What we're going to do this afternoon is we're going to have an opening talk on the current status of Gulf War exposure data. That will run for about 30 minutes. Then we have a series of experts that are going to spend just a few minutes telling you what they do, but we really want to make this a discussion to discuss the issues regarding the methodologies we use here. So, I'm going to ask them to talk for just about 5 minutes and tell a little bit about the work they do, and for each of them to define what they think is a critical methodologic question to address. And then we'll start a panel discussion.

So, before we get into that, let us start with Jack Heller who is going to do the opening presentation. Jack is a senior scientist in the Deployment Environment Surveillance Program,

U.S. Army Center for Health Promotions and Preventive Medicine. He has oversight responsibilities for issues related to troop environmental exposures that occurred in the Persian Gulf region during Operation Desert Shield/Desert Storm. He has a doctoral degree in insect physiology from the University of Florida. The program, the Deployment Environmental Surveillance Program, incorporates all aspects of the Persian Gulf exposures which DoD uses to identify potentially exposed troop units. Also included are the Operation Joint Endeavor, environmental monitoring, health risk assessment efforts, and the development of a new system that will better track troops and determine potential exposure that may have an impact on their health. What Dr. Heller is going to talk about today is the current status of Gulf War exposure data. Dr. Heller.

Jack Heller, PhD
Senior Scientist
Deployment Environment Surveillance Program
United States Army Center for Health Promotion and Preventive Medicine
Aberdeen Proving Ground, Maryland

Current Status of Gulf War Exposure Data

Thank you. Good afternoon. We've heard a lot of really good talks the last day about some of the effects of chemical exposure on the body. What I'm going to discuss is what we know about the exposures veterans had during the Persian Gulf War.

Basically, if you look at the PAC report or any other reports that have been done looking at Persian Gulf exposures, it's the same list that generally comes up over and over that are environmental exposures that occurred in the Gulf, or other stressors veterans were exposed to that potentially may be contributing to veterans' health problems. I'll try to discuss those one at a time.

Oil well fire exposure. This is a data base I think we have more data on than any of the other ones. We were tasked by the Surgeon General of the Army to go over and look at the exposure of troops in the Gulf to oil well fire exposure. Before we got there, there was a lot of preliminary exposure monitoring done, although not extensive, by the U.S. EPA, the French, and the Kuwaitis. The analysis of that data showed no acute threat from oil fire smoke, and that's what their data showed. Since we were going to have troops there a longer period of time, we were concerned about what chronic effects might have been to a longer term exposure. So, we deployed to the Gulf at the beginning of May of 1991. We wanted to get their sooner, but most everybody was leaving theater and it was difficult to get in. So, we didn't get there until May 1st, and unfortunately, we weren't there for the first 2 months of the oil fire. When we got there, there were still about 580 oil fires burning, and we did monitoring the entire time the oil well fires were burning, and a month after for background data.

Our charge was to try to do sampling where we had major troop populations for long-term. So places like Khobar Towers where the terrorist bombing occurred, we had a large number of troops there. So, even though that was fairly distant from the oil fires, we did a lot of monitoring there. Again, in Kuwait, we did it in major places where we had U.S. Forces. As you can see, some were very short-term monitoring efforts because some of these places closed down after we got there, and a site would be transferred to where we now had troops. We had sites as close as the Ahmadi Hospital which was about a mile from the Ahmadi oil fields that were burning, and as I said, to as distant as the Khobar Towers.

We collected over that time period about 4,000 ambient air samples, about 200 surface samples, and about 600 industrial hygiene samples or from personal samplers warn by troops or very near where troops were. We tried to look for a wide variety of environmental contaminants. We looked for about 53 different contaminants, volatile organics, polyaromatic hydrocarbons, some of the acid gasses, some of the criteria pollutants like ozone, sulfur dioxide, nitrogen dioxide. And then we looked at the particulate matter, both the respirable particulate matter 10 microns and under and total suspended particulate matter. As was mentioned before, people are more concerned with 2.5 or 3 and below, and that's the new standard EPA's promulgating. At the time we did this in '91, the standard for respirable particulate was the 10 microns and below. So, we have 10 micron and below, and then total. Associated with the particulate matter, we have heavy metal data.

Following the Gulf War, a couple of public laws were enacted, 102-190 and 102-585 that mandated that we determine each individual Gulf War veterans' exposure to oil fires, how long they were exposed, and what the extent was. Well, we had 10 sites around the theater, we weren't over the entire 880,000 square miles, and we missed the first 2 months of monitoring. So what we had to do was re-create that. So, we worked with NOAA, the Atmospheric Research Laboratory. They have a model called Hysplit which is used for monitoring large sources. So, we used the NOAA Hysplit Model, along with the National Center for Atmospheric Research's satellite imagery, the NOAA oil fire extinguishment chronology, pollution emission factors from those oil fires, and other things to recreate exposures at places and times we weren't monitoring.

These are the things we modeled for. You saw, as I said, a larger set of heavy metals we looked for when we sampled, but when we did our modeling for oil fire exposure, we would only do three metals – nickel, vanadium, and iron which are contaminants of Kuwaiti crude. So, this is a smaller subset of the larger set.

So, what we would do on a daily basis is, we'd take our satellite plume, which would show us where the plume was, and then on that same day, we would do modeling which would give us concentrations at over 40,000 grid points in the theater, and basically merge those 2 together with a safety boundary around it to determine if a troop unit was under the oil fire smoke and should get exposure.

Now, to determine where troops were, which is key to getting good exposure data, what we first do is put out a base map on our region of interest, and then we use a database constructed by the Center for Research of Unit Records. And they basically took about 5 million different records, troop logs, situation reports, that said where a unit was on a particular day and what that latitude and longitude was so we could identify where a troop unit was.

Following that, we would go to the Defense Manpower Data Center's Persian Gulf registry. And so we would have a unit identification for a particular troop unit, we'd know how many people were in it, and we would then be able to identify what individual exposures were or were not for a particular individual. Now clearly, some people weren't always with their unit. We have days when there were multiple locations for a unit. Of course, a unit may have moved. So we had to make adjustments where we would look at what the maximum possible exposure would be, or the minimum possible exposure would be, depending on their location during that day.

So, what we're now able to do is look at a unit and find out their excess cancer risk or non-cancer risk. So we have a program, we can show that the top 1 percent of exposed units. So, we can for the units over there, finally, look at that exposure. Now, that just gives a risk level and we want to go further than that. So what we want to do for an individual, and this is a social security number of a particular individual, is be able to look at what that individual's exposure and risk was from each of the individual compounds we've looked at. So, we're now just starting to be able to look at it for individual compounds, both modeled and sampled risk. So, the part contributed by the oil fires, which is just modeled, or total risk which is what we have from our sample risk. And on this printout, you would get what the mean soil concentration would be in the area a troop was, what the mean concentration was for the particular compounds. So, it gives a pretty good look at what a veteran's exposure may have been.

We also have some direct measures of exposure where we did what we called the Biological Surveillance Initiative. We had a group in June, 1991 who deployed from Germany, the 11th Army Calvary Regiment, and we took blood and urine samples, and did questionnaires, and actually measured what their pre-, during-, and post-deployment measures were for certain compounds. And as I said, we did questionnaires, serum metals, serum volatile organics, pH DNA adducts looking for pH concentrations, and sister chromatid exchange looking for genic stress. Now, we did not do all this work. The blood volatiles were done by CDC. Serum metals were done by Armed Forces Institute of Pathology. National Cancer Institute, I believe, did the DNA adducts. Melissa McDiarmid who is the chair of our work group did that work. And so we had a diverse group doing that.

That's exposure one, oil fire exposure. Next was chemical warfare exposure. The biggest exposure we know about was the demolition of the Khamisiyah pit. These are the 122 millimeter rockets, crates, and this is the pit following detonation. We have really no monitoring data on what the levels were, so what we had to do is go and model what the exposure was. So what we

did, working with the CIA, Defense Special Weapons Agency which is now Defense Threat Agency, Naval Surface Warfare Center, NOAA, we gathered the best information we could about how many rockets were at the site, what the concentration of sarin and cyclosarin was in them, how the rockets were blown up, how much would have been destroyed by the explosion, how much would have been blasted into sand and wood and volatilized over the day to come up with a prediction of, on a daily basis, where the exposure levels were. We had the first noticeable effects level which would have been from the first very minor effects of dilated pupils, runny nose, all the way to death. And then we had a lower level exposure which is from that point out to the general population standard. These were used by the Office of the Special Assistant to notify people who were potentially exposed to chemical agents. We are modifying those, we have better met data, we've improved our model. So, we're going to be putting out new notification plumes to re-notify some of the veterans. So, that was day 1. This is the day 2 plume. And again, we notified these people by seeing what the troop unit was under the plume, and then who was in that region. That's day 3, and day 4.

One of the other things we're doing is we have what's called the epidemiologic plume. And that has been selected by an independent peer review panel of university researchers, other government researchers, from National Oceanic and Atmospheric Administration, and other scientists. What is the best model to choose to study an exposed cohort of troops versus a non-exposed cohort of troops? And this is just the plume we get for what selected as the best model. This study is going to be done by the National Academy of Sciences Institute of Medicine when we get the latest plume model.

This is what we call an ensemble model. So, as I said, it's made up of the intersection of various meteorological weather models and dispersion models which track how the agent would move through the environment. So, it's a very conservative and inclusive way to look at exposure, and that's what I think the PAC said and the Office of the Special Assistant wants to do in notifying people who were potentially exposed.

The other CW efforts, there are 3 other sites from the air campaign that may have been bombed at Ukhaydir, Al Muthanna, and Muhammidiyat. And we're working with the CIA now to get what the source term would be. In other words, how much agent was there and what agent. And with the models, again, to try to come up with where these contaminants, these chemical agents, may have gone, and what troops were exposed. There are also some cases at OSAGWI, Office of the Special Assistant on Gulf War Illnesses, case narratives on individual smaller exposures and some work on chemical detections.

This is just a map that shows the Fox vehicles and M256 detections throughout the Gulf War. And so we have the day when that detection occurred, where it occurred, and if we wanted to do a study with looking at troops that were around single detections or multiple detections, we can do that. So this is some more of the potential exposure date we could use.

Particulate data. One of the biggest, I think, the biggest exposure that people got in the Gulf War were to particulates, both oil fire and sand particulates. So, in the way of hard data we have, as I said, 590 total suspended particulate samples and about 244 respirable particulates to look at the level troops were exposed to in various regions of the Gulf. Again, we have our model data that showed us what the oil fire exposure data was. This total data is a combination of sand and oil fires. So if we wanted to single out oil fires exposures, or a particular exposure, this is what we use – the NOAA Hysplit Model and the NCAR satellite imagery. As I said, we have sand data from 10 sites over 7 months. Well, troops were in the Gulf in areas we didn't have sampling and for periods before that. So, we're working with NOAA to develop a model that can predict what the sand exposure would be in any area of the Gulf any time troops were there. And we're using historic data from the Kuwaitis and the Saudis, plus doing some re-analysis of our particulate air samples and our soil samples looking at grain size to come up with the parameters you need to do that model. OSAGWI we'll be releasing, fairly soon, a health effects study case narrative on what sand exposure meant to Gulf War veterans. And it's based on, again, a lot of the historic data and a lot of the sample data we have.

This is, you know, kind of what we can do on a daily basis if we want to look at what a troops' particulate exposure is to oil fire. We can model what a particular exposure level in micrograms per cubic meter of oil fire particulate, see where that troop was on a particular day, to come up with exposure to do epidemiologic studies with.

DU environmental data. Again, another potential source of exposure. We do not have good environmental data from the Gulf War that was collected, so what we are basically doing is trying to reconstruct what that exposure would have been. Now, we have a lot of data on, test data from test ranges that was done by DoD and non-DoD organizations that look at, you know, external dose measurements, DU munitions striking targets, fires in vehicles loaded with DU munitions, personnel and vehicles struck by DU. So, to get and assess the DU exposure, since we don't have data, we're going to have to model that exposure based on the data that's been done in the past and continues to be done.

Again, we've done tests on the test ranges on dispersion, aerosolization, resuspension, and environmental fate of depleted uranium when it's been buried in the sand. The sampling that we have done, that's been done by DoD, has been done by us through the 520th Theater Army Medical Lab, and that's been basically surveys for more or less other reasons. So what we have are the air samples. We did 200 air samples in '91 for gross alpha and gross beta. Now we didn't do depleted uranium, but DU is an alpha emitter. So it was just for gross alpha and gross beta. We have 610 samples remaining that we're going to try to develop some methods to try to be able to assess them for DU. We've done some samples on destroyed Iraqi tanks in the Iraqi tank yard in the Udairi Range, and again, they were gross alpha and gross beta measurements.

We had a lot of soil samples that we collected to look at oil fire exposure. But again, we have

154 samples from 1991, 94 from '93. What we're doing now is isotopic depleted uranium analysis to look at both total and depleted uranium. We have also some samples from '96. In 1998, we have 22 samples, these 2 should be reversed, where we actually did isotopic depleted uranium analysis. And we're going to continue that to try to get a better data base. Now, these are again the sites and the years where we collected those samples around the theater. Unfortunately, we don't have samples from where some of the tank battles were, but we do have . . . [a gentleman from the audience indicated that he had a sample collected from a tank battle site which he would be willing to give to Dr. Heller]. Well, okay. We're willing, I mean, that's the thing, we're willing to take samples and look at people's samples. And again, we've always been willing to share our data and our samples with other researchers as we've done with NOAA who has done analysis. I wish we had more samples in areas where actual tank battles had been fought. We don't have that at the current time. It was mainly on troop locations.

These are the locations where we did our sampling in 1998. Again, in some areas where it would have been suspected you would have had high depleted uranium concentrations on the Udari Range, the Iraqi tank yard, and the highway of death where there were a lot of destroyed vehicles that were killed with A-10s when the Iraqis were retreating from Kuwait.

DU health effects data. We have toxicological and radiological studies that have been done on uranium miners, and there's been some work in animal models, we have it with natural, enriched and depleted uranium, and multiple forms. The health risk is determined by the amount of depleted uranium that's internalized.

Again, there have been some assessments of the health effects of depleted uranium. There's a lot known about depleted uranium, probably more than most of the other environmental exposures we're looking at. The BEIR study by the National Academy of Sciences, and there's an ATSDR toxicological profile, and I believe another on is about to be released on depleted uranium. Latest research on retained depleted uranium fragments by the Armed Forces Radiobiology Research Institute, and then I call this, it's not really research, it's patient monitoring and surveillance being done by the Department of Veterans Affairs, Melissa McDiarmid, and development of a biokinetic model for uranium contaminated wounds by Oak Ridge. And finally, our organization, USCHPPM for the OSAGWI is looking at probably 12 exposure scenarios from being in a tank that was hit by depleted uranium, to maintenance workers who went on tanks that had been hit, to people going by burning tanks to estimate what exposure would be, and right now to determine the adequacy of the published data for estimating suspension or resuspension, because again, this is going to be a modeled effort. We're going to evaluate the strengths of the data for modeling, and recommend other work that needs to be done to give us all the data we need to do good modeling for depleted uranium exposure.

BW air sampling. Again, I didn't know this until I talked to the people at the Office of the Special Assistant for Gulf War Illnesses, they're doing a case narrative that will be coming out on BW. I

guess more than about 900 samples were collected by U.S. forces, chemical and biological defense teams. There were no confirmed detections of anthrax or botulinum toxin in these 900 samples. We have some very limited soil data, 2 samples taken at 12 different locations. That was sent to Fort Detrick U.S. Army Medical Research Institute of Infectious Disease and there was no evidence of BW agent found there. That being said, obviously, there were a lot of other places in the theater where we don't have samples from. That OSAGWI case narrative will hopefully be coming out soon that will talk about all that. And these are just where the different teams were who did the air sampling at night around the theater.

Vaccines, again, another contentious issue. We know anthrax was given to about 150,000 troops, bot tox to about 8,000. The problem is – who? There exist partial records for probably about 7,100 people who received anthrax and bot tox. But, out of all these people, that's all the records we have of an actual social security number and what inoculation a veteran may have received. And, obviously, there were all the other routine vaccinations that you would have gotten when you deployed unless you were already up-to-date for them.

Pesticides, another contentious issue. Not a lot of data on this. All I can say is OSAGWI is doing a case narrative where they're going to be trying to work with modeling exposure. Again, we know what pesticides were ordered to go to the Gulf and they were DEET, permethrin, some of the organophosphates, malathion, etc. Beyond that, we don't know much. We don't know if that was all shipped to the Gulf or not. OSAGWI is doing a very extensive investigation to try to determine if this is one of the, I would say I guess, our weaker exposures because we just don't have a lot of good data on it.

Pyridostigmine bromide. Again, we have an estimate by DoD that 250,000 personnel took at least one tab. Again, it's who took it, really how many they took. Again, not a well documented exposure data base. There is a RAND literature review on pyridostigmine bromide, and there are some toxicological studies on the synergism of DEET, permethrin and pyridostigmine bromide – one that our organization has done and one that was done by Dr. Mohamed Abou-Donia who gave a talk this morning from Duke University. Again, on GulfLink, there are a number of case narratives that the Office of the Special Assistant has done on various aspects of some of these many exposures we had. And there are a number pending – the one on pesticides, the one on BW, I believe there's going to be one on CARC paint, one on exposure to water over there. Again, because we don't have a lot of data on all of the different sources of bottled water that was consumed in the Gulf War by our veterans.

I guess that's it. So, that was not bad for all that, all that data. That's kind of at least the state-of-the-art of what I know about, you know, what's been collected on Gulf War veterans' exposures.

Edward Bryan

Malden, Massachusetts

The Office of the Special Assistant isn't doing enough. He is doing nothing. I just want this audience here to know that office is doing nothing and your office isn't doing too much on the pesticide exposure or the oil well fire exposure. As a fire fighter . . .

Mr. Albert Donnay

Dr. Heller, could you comment on tent heater emissions?

Dr. Jack Heller

Oh. I left that out.

Dr. Stephen Thacker, Moderator

Excuse me. We have about 5 minutes that the audience can ask questions of Dr. Heller before we get to the others, as the other panelists come up. So go ahead with your question.

Discussion

Dr. Jack Heller

The tent heaters. A tent heater study was commissioned by the Persian Gulf Veterans' Research Board that's being conducted by Loveless Research Laboratory in Albuquerque, New Mexico. I'm not sure of the status. I tried to get in touch with the principle investigator before I came down. I know it should be probably pretty near complete. And they were looking at different tents, burning different kinds of fuels, I guess in vented and unvented tents. I really don't know the status of it.

Mr. Albert Donnay

I know the status of that. I have a copy of it, and I'd like to inform this group because the report to Congress is very misleading. As with many of the abstracts in the report to Congress, they were not written by the investigators. The investigator told me that, although the report is still entitled *Studying the Emissions of Unvented Tent Heaters Using Leaded Diesel Fuel*, and that's what was funded, when he tried to begin the program, the Army told him that no leaded diesel fuel was used in tent heaters, and that there were no problems with military tent heaters, and that he should therefore study the kerosene in jet fuel used in civilian heaters, which is what he did. There is no assessment of health effects in this. They were just trying to model the carbon monoxide and other emissions. But, we're very frustrated to see that, in fact, there is still no

study of the diesel emissions, and I urge the etiology group to recommend further research in that area. Thank you.

Dalia Spektor, PhD
RAND Corporation
Santa Monica, California

I want to bring to your attention that RAND is also in the process of starting a very extensive survey questionnaire on veterans regarding pesticide use. And we expect to get some at least levels of exposure, not exactly for everybody, but some maximum levels that people were exposed to in different situations. And the other thing I want to say is that next week, close to here, there is a Health Effects Institute Diesel Workshop where some of these issues will be discussed on the health effects of diesel fuels.

Joseph G. Miller
North Carolina National Guard
West Jefferson, North Carolina

My question is, do you believe the information given, the numbers of amounts of agents and stuff released, that were released in 1996 I believe, from the Presidential Advisory Committee? Do you believe those numbers to be accurate?

Dr. Jack Heller

I'm not sure of the numbers. From Khamisiyah?

Mr. Joseph Miller

Well, from Al Muthanna, and Mahammidiyat, from Khamisiyah. From all 3 of them. Do you believe those numbers to be accurate?

Dr. Jack Heller

If they were the numbers that they got from the CIA and DoD, I have no reason to believe, you know, they're not accurate.

Mr. Joseph Miller

Are they going to change the numbers or just the mapping?

Dr. Jack Heller

Okay. Well, oh, that I can discuss. They have a good handle, and they always had a good handle with Khamisiyah on what the source term was. They knew how many rockets were there, so I think if you're concerned about Khamisiyah, the source term is good. What they've worked on, and worked on improving are the meteorological models and the dispersion models. So, they're not changing the source term. I think they're working with getting better meteorological data. They're working with, I think the first time it was done, there was no degradation factor put in for agent, which we know happens. So, the modeling and the models are what's being worked on. I know the source term is fairly well established from Khamisiyah, and I know we're still working with the CIA to work on the source terms at the other three sites. I don't know if they're as well established or not. Which is how much was there.

Mr. Joseph Miller

We're still talking about 8.5 metric tons at Khamisiyah in Bunker 73?

Dr. Jack Heller

No. We're talking about the pit. How many rockets was it?

Mr. Joseph Miller

1,809.

Dr. Jack Heller

1,800 rockets. Right.

Mr. Joseph Miller

No, actually, the pit was 1,250 some rockets, and it was 1,882 gallons. But according to the information that you sent me a couple of weeks ago, the total release in the plume, when you do the mathematics and do all the subtraction, it was down to 350 some gallons, I believe.

Dr. Jack Heller

Right. What happens is a certain amount was destroyed by the explosion, certain amount went into the water and into the sand, a certain amount volatilized off, right. So, the total that was left sounds about right, but I don't have the figures in front of me.

Mr. Joseph Miller

I didn't mean to bother you or contradict you, but I've got a lot of this information on my website, and I'm trying to make sure I keep it correct because there's been so much misinformation put out, I'm trying to keep it as close to what we actually know as we do.

Dr. Stephen Thacker, Moderator

That's all we have time for at this time, but we're going to have time at the end for people to ask further questions.

Craig Stead, MS, MBA
Putney, Vermont

I would like a chance to ask a question, because I have yet to get to ask a question in these proceedings?

Dr. Stephen Thacker, Moderator

If you make it brief and identify yourself, you may.

Mr. Craig Stead

This is for Dr. Heller, and what it is, is the veterans' report massive exposures to smoke in the oil field fires based on plume touch down which was related to air inversions and very low wind speeds. This is going to be the first question, I'm going to throw the second part in here. They also reported massive exposures to petroleum, both crude oil raining out of the sky, oil in the water, oil in the – let's say drinking water and shower water. The first question is, most computer models I've seen of air pollution do not allow for micro-meteorological conditions such as air inversions, zero mile an hour winds, and plume touchdowns. The first question is, does your model incorporate these exposures the vets have reported? The second question is, petroleum is a known carcinogen, toxin, it causes respiratory problems, and skin rashes, and many other toxic expressions and symptoms. Why have you not included any exposures to petroleum in your health risk assessment which finds no adverse affect? Would you please answer both of those?

Dr. Jack Heller

Number one, yes the model NOAA used does ground level concentrations. In other words, the level we use is the one that's at the breathing zone.

Mr. Craig Stead

But does it include plume touchdown is my question? Which is why we have high concentrations that are like up to 10 times what is considered a significant harm level by the EPA. These have been reported by the WHO. My question is, does your model incorporate this kind of exposure of the individual?

Dr. Jack Heller

Well, all I can say is, it calculates, using the best meteorology they have, what the concentration is at the breathing level, at the 2 meter height.

Mr. Craig Stead

But that doesn't answer my question, but you're telling me that it does. Could you please comment on petroleum then so we can get on with the program?

Dr. Jack Heller

You're right. I have not looked at dermal exposure to petroleum.

Mr. Craig Stead

How about inhaled exposure?

Dr. Jack Heller

Or that. We were looking at oil fire smoke and soot from oil fire smoke.

Mr. Craig Stead

How about contaminated water? Oil contaminated shower and drinking water.

Dr. Jack Heller

Again, I haven't looked at that. Can I make a couple of comments?

Mr. Craig Stead

So those three exposures have not been incorporated into your model.

Dr. Jack Heller

No, no, what you need to do, and that's clearly stated in there, it is. I mean, I tell what we looked at and what was assessed. If you want to look at people who bathed in oil, who got those kind of exposures, look at the oil firefighters. There's been a lot of work done by them, and I can never remember, I know it's Dr. Friedman, and he has done a lot of work on them along with some blood volatile work done by the CDC. Again, we kind of used that as a surrogate, because clearly, they were exposed to much higher levels, dermal and inhalation of petroleum than most of our veterans. Again, some of them were exposed to oil rain, if you will, and they got it on their skin, they inhaled it. Again, we didn't have good data to do that exposure and we did not include it in our risk assessment. We didn't try to say we included it in our risk assessment. Again, the water was bottled. So, hopefully, most of the drinking water didn't have oil.

Mr. Craig Stead

I posted a question from your report to the Gulf War e-mail pages where you said that you would not consider water as a route of exposure because all troops were provided pure bottled water. I posted that as "Is this true?" And what came back was 24 comments of "No, the water was contaminated in the showers. The drinking water was contaminated. They hauled the water in fuel tankers. The desalination units were by-passed by the oil slick and it was in the water from the desalinization units." This is the veterans reports. This is not a computer model. That's all I have to say.

Dr. Stephen Thacker, Moderator

Thank you Mr. Stead. I'd like to move on to our next presenter. John Feussner is the Chief Research and Development Officer of the Office of Research and Development for the Department of Veterans Affairs. He directs and oversees one of the largest federal research programs dedicated to health care research. He got his MD degree at the College of Medicine at the University of Vermont, and is diplomat of the American Board of Internal Medicine. At the Duke University of Medical Center, Dr. Feussner is professor of medicine, and was the chief of the Division of General Internal Medicine from 1988 to 1996. In 1996, Dr. Feussner was the first recipient of the Mark Wolcott Award for clinical excellence for exceptional service in providing outstanding clinical care to our nation's veterans. Dr. Feussner.

Research Strategies (Panel Discussion)

John Feussner, MD
Chief Research and Development Officer
Office of Research and Development
Department of Veterans Affairs
Washington, DC

Thank you Dr. Thacker. Good afternoon. I've been asked to make some brief comments, not a formal presentation, and I've been vacillating about where to start. I think where I'll start is first to make an observation of my own about the conference, and then second to make a comment about the federal research effort. The first is a simple observation. From my view this meeting has been a scene of contrast between the simplicity and the directness of some of the questions that the patients have asked, and the complexity and the difficulty of the research seeking to address those questions. The questions that the veterans asks are clearly important, and deceptively simple. Quite frankly, they're the same questions that many of my own patients have asked me on numerous occasions, "What is causing my illness?" "What is the diagnosis of my condition?" "How can I be treated?" "Make my illness symptoms go away."

The research paradigm is similarly deceptively straightforward. There are just 3 essential components. What is the research question we are about to address? What are the methods that are most appropriate to be applied to that question? What do the data say, and are the data analyzed appropriately? For the research method itself, there are only 3 basic components. Defining the patients we want to study or the problem we want to study, establishing what the exposure is or in the case of trials what the intervention is, and then measuring the appropriate outcomes. This sounds straightforward and simple. For an exposure, all we want to know is what is the exposure, and then what's the dose, the duration, and then the interactions of the exposure? Simple questions. For an intervention we ask the same kinds of questions, and in addition want to know about side effects or adverse effects of the intervention and compliance with the treatment. So, like the questions that the patients ask us, the research is deceptively simple. And, as you've heard just recently, and throughout the days, the difficulty is in the details.

Now second, I'd just like to make a brief comment about the federal research program. I believe it can be expressed in a straightforward way as three complementary activities. The first component is a broad, fundamental research effort to understand the basic biology and the basic science surrounding the multiple exposures that were possible in the Gulf. Most of the research that you've heard presented at this meeting has been funded through these federal research programs. On the positive side, the research focuses clearly on understanding the basic mechanisms of disease. For example, how a single exposure might have multiple or heterogeneous effects on some patients. On the negative side, this research is difficult, it is painstakingly slow, and it produces results only incrementally. The second component includes major epidemiological research projects frequently studying thousands and even tens of thousands of veterans. Given the difficulty and the complexity of these studies, it is not surprising at all that they have limitations. Large scale studies cannot investigate patient illnesses in great detail. So, measurement compromises must be made. Small epidemiological studies can make more detailed patient measurements, but the patients are highly selected. The study is not population based, and the results may not applicable to the majority of veterans. And, there's another difficulty. That is, the delayed effects of some of the exposures. We know that even if soldiers have experienced single and multiple exposures, they may not be experiencing diseases for years to come as the

damage done may be latent and takes time to express itself as disease.

Mr. Hirst from the VFW indicated earlier that his son is a healthy Gulf War veteran with several healthy children, but he's wondering whether his son is going to stay that way. Our large population based epidemiology studies looking at disease and death rates will address his concerns, but it will take years. It will probably take decades to complete that work. In the mean time, with the basic research producing results incrementally, and the epidemiological research taking years to complete, what are we doing? This brings me to the third component of the federal research effort. We are initiating major national treatment trials to study the effects of antibiotic treatment and patient centered treatment strategies. While the basic and epidemiological research is ongoing, we're implementing treatment trials to study whether new or different treatments can help our patients get better.

We know, I know, you know that nobody wants to be sick. Even more, nobody wants to stay sick with no hope in sight. We need your help with these trials. The faster we can enroll veterans into these treatment trials, the sooner we can determine whether the treatments work or not. And if they work, how well they work and for which patients or who the patients are that they help. I think I will stop. Thank you.

Dr. Stephen Thacker, Moderator

What I'd like to do is let each of the panelists give us a perspective of their scientific work and, so we'll just move along. Next is Gary Gackstetter. He's a colonel in the United States Air Force Science Corps. He's also assistant professor and deputy director of the Division of Epidemiology and Biostatistics, Department of Preventive Medicine and Biometrics, Uniformed Services University of Health Sciences. He is a doctor of veterinary medicine, with also a master's degree in public health from the School of Medicine at Boston University, and a doctoral degree in epidemiology from the University of Minnesota. He recently served the Pentagon in the Office of the Assistant Secretary of Defense for Health Affairs as senior policy analyst of epidemiology, and then as the deputy director for military public health within Clinical Services. He serves as DoD's senior expert in epidemiology for all clinical and scientific research matters.

Gary Gackstetter, DVM, MPH, PhD
Colonel, United States Air Force, Biomedical Sciences Corps
Assistant Professor and Deputy Director, Division of Epidemiology and Biostatistics
Department of Preventive Medicine and Biometrics
Uniformed Services University of the Health Sciences
Bethesda, Maryland

Thank you. I'll try to keep this very short. I'm going to try to do methodologically what an epidemiologist really looks for. You really only need 3 things. You need a high quality case

definition so that each case is very specific. You need high quality exposure information, and if you have exposure and case information, you can look at associations. On top of that, you need a perfect or an appropriate comparison group or control group. As long as you have the control group, great exposure information, and a very clear, crystal clear case definition, things become very easy with epidemiology. And thus the rub. We have less than ideal, soft, let me say that a little differently, gray areas as far as trying to define a very specific case. We don't have crystal clear perfect dose intensity kinds of exposure data. And our comparison group is probably going to have to be drawn from within those that were deployed. There is no comparison group, a perfect comparison group among those that were deployed to the Gulf.

So, when you really boil epidemiology down to those 3 things, you see what a challenge it becomes to look at the illnesses in Gulf War veterans. Nobody's saying that there aren't illnesses. What we're saying is it's very difficult to track this very complicated problem, to put the puzzle pieces together so that we can understand the big picture. And I'll stop there.

Dr. Stephen Thacker, Moderator

Thank you. We've also asked people from outside the government to work with us in this, and I'd like to introduce Dr. Robert Haley. Robert is the Director of the Division of Epidemiology, Department of Internal Medicine, University of Texas Southwestern Medical Center. He worked 10 years at the Centers for Disease Control before moving into the academic world. Since early 1995, Dr. Haley has devoted his research effort to understanding the epidemic of neurologic illness affecting Gulf War Veterans. He has published articles in scientific journals identifying syndromes and linking them to neurologic damage and risk factors of wartime chemical exposures. Dr. Haley.

Robert Haley, MD, FACE, FACP
Director, Division of Epidemiology
Department of Internal Medicine
University of Texas Southwestern Medical Center
Dallas, Texas

I think Dr. Gackstetter well described the objective of the epidemiologic studies. Here's what I think happened that derailed this effort about 1995. There was a, and let me not be unkind, this is an extremely difficult activity that all of the participants have been involved in, and we should not find villains. But, this really did go wrong in about 1995. There was a proposed case definition developed by Dr. Jay Sanford within the Department of Defense in the '93 - '94 time frame. That was proposed to be the basis for doing some epidemiologic case control studies to try to determine the nature of the illness and perhaps relate it to risk factors in the war. Something happened. I don't know what happened. But that was derailed. That was sidetracked. No case definition was come up with, and instead a policy was formulated to say that there is no single

illness, no case definition could be developed because that would admit that there's an illness. So, case definitions were basically barred. No epidemiologic studies were done. Instead what happened, we had large population studies, computer studies. You saw the 3 studies in the *New England Journal* and you perhaps read my critique of those in the *American Journal of Epidemiology* looking at the healthy warrior effect and the unequal follow-up. What happened was, without a case definition, they looked at all 600,000 people who went over compared to about an equal number who didn't go over. But without a case definition, they tried to look at differences in mortality, hospitalization, and birth defects. The problem is, the people who were exposed within the deployed group was probably a relatively small group, maybe 15 or 20 percent. And that's actually very large, but statistically it's very small. And so any difference between these 2 populations due to that exposure is washed out. Moreover, think, who we send to the war? What kind of soldiers get sent over there? It's the healthy soldiers that get sent over. The sick people have to stay behind. Well, where are they in the comparison? They're all concentrated in the non-deployed group. So, therefore, the non-deployed control group is sicker than the deployed group. So you see, after the war, if you see equal rates, that means something happened to the guys who went over and it made them as sick as the ones who didn't go over. That's what we call the healthy warrior effect. That was not controlled for.

The first speaker in the conference, I was shocked, presented those same data as if those were still accepted and they're not. Those are highly biased and they've seriously under called the health effects, certainly hospitalization, and certainly mortality, and possibly birth defects – we're not sure about that. Also, following up only in military hospitals means that people who left the service right after the war (i.e., who were too sick to continue serving), they're not being followed, so we've preferentially excluding them from the follow-up. So, therefore, any birth defect studies or the hospitalization studies are seriously under calling.

Now, it turns out we still see some differences. We still see that the deployed troops had more serious hospitalization and mortality, so that means these have been seriously under called. Let me just say one more thing. What should have been done is what I think we did. Now, it sounds self-serving, but we came into this in 1994 with private funding and said, "Well, now what shall we do? What would CDC have done if CDC had gotten involved in 1993?" It's what they would have done with any epidemic investigation. They would have gone through the CDC epidemic investigation fire drill which is very different from what's been done. Toxic shock syndrome, Legionnaire's disease, HIV, Hanta virus, a thousand other epidemic investigations over the last 50 years have all been done this way. You go in and you examine a dozen, twenty, ten – a number of typical cases – find out what they have in common and you write down a case definition. Now, there's an old saying at CDC. The first step is to write a case definition. And if you can't, then write a case definition because failing to do so means that you will necessarily miss the effect. You will not find anything because of not having a case definition.

There still is not a case definition, and that's the reason everything's been negative. What we did

is we went to a group of Seabees, it was arbitrary, we went to them because we could find them, we surveyed their symptoms, put them in the computer, did a factor analysis which comes up with different groupings – we don't know if those were right, but those were arbitrary, it was a case definition. I mean it's not arbitrary, they were statistically done, it's a case definition. We then compared those to exposures. We brought them in and did neurological tests on those people who met the case definition and those who didn't. And sure enough, they were done blindly so the doctors didn't know who was who, sure enough, the guys who had these symptom complexes, they had brain dysfunction compared to the normals. We then looked at their risk factors, and sure enough, we found relative risks of 4 to 8. Now, we were accused by some very vitriolic, VA and DoD accusers, who accused, who said this is due to recall bias. I defy anyone to find any study anywhere where recall bias has produced a relative risk of 4 to 8. They're usually in the range of 1.5 to 2 if they're there at all.

So, we think we came up with some very important findings. They've been basically neglected. We've been unable to get grant support through the peer review process. Everything we've submitted, we've submitted 5 grants including one recently to the VA to do a treatment study. The VA central office kept our proposal for 8 months, a study to do n of 1 trials, and hundreds of veterans in the Dallas and Temple VA collaborative hospitals. It sat at the VA central office for 8 months unreviewed. We finally, after inquiring 20 or 30 times, finally some low level person said, "Sorry, you were not funded, you're not going to get anything in writing. There were no peer reviews, there were not pink sheets, it's not what we wanted." The point of this is, there is a way to do this research. It is not long, it is not time consuming, it can be done relatively rapidly. What we should have is a series of 20 or 30 case control studies by the best epidemiologists in the country. Those should be funded immediately. They should all develop their own case definitions, see what's true in that whatever little group they're studying. Then we ought to aggregate those and do a random sample survey to look at the external validity of whatever those findings are. This has not been done. I think we're just dragging out feet.

Dr. Stephen Thacker, Moderator

Thank you Dr. Haley. Sitting in for Dr. Penelope Keyl is Dr. Rebecca Bascom. Dr. Bascom is professor of medicine at Penn State College of Medicine. She has particular interest in practical approaches to developing and testing treatment.

Dr. Rebecca Bascom
Professor of Medicine
Pennsylvania State College of Medicine
Hershey, Pennsylvania

One thing that's always important to know is to know what you don't know. And I know that trying to understand what the proper design is for an epidemiologic study in a certain

circumstance is something that I have difficulty understanding. I was very impressed by what I just heard, but I also know that I suspect other people would disagree. And what I think is very important would be to hear a debate of epidemiologists going back and forth on this issue. Because I think the credibility of the epi studies is something that's pretty important to have an open debate and discussion about. I do think it has been disappointing not to have more epi analysis of the poorly defined syndromes, and I think that's something that should be discussed and critiqued because some people thought that couldn't be done for good reasons. I'm hearing you [Dr. Haley] say you think it could and should be done. I'm not an epidemiologist. I'd like to hear that debate.

That being said, I am a doc. I take care of patients. I do clinical research. I try to understand how to make progress. Dr. Keyl and I worked together when she was interested in coming up with practical definitions to move forward. Here's what I think. I think that we have about 3 problems. One is we've got a bunch of people who served our country and who are now not back to where they were before they left, and I think a warrior makes a crummy beggar. And I think some part of the dynamic that has developed is that warriors are being put in the position of being beggars, of being dependent and trying to beg for what they need. In occupational medicine, that's a very classic and hurtful dynamic to have in someone who has been injured. Addressing that dynamic is real important. So, I think methodologically, there are people that know how to look at situations like that and to try to get at them. I would say, I know it sounds soft, but people that know how to make a good organization need to take a look at how the VA and the vets are dealing with each other and see if some of that can be worked on. That particularly involves how compensation is handled and the lack of redress. Basic worker comp in the non-VA environment, non-military environment, says that there is an appeals process. And, you know, it's messy. But, it does have a process so that your employer and your worker comp insurance company is not the one that's making the call as to whether or not you should get compensation. So, I think that's a research question actually – to not just look at the individual treatment, but to look at some of these big picture things.

Second, I think the VA needs to move quickly to develop rehabilitation modules that work for Persian Gulf era people. I think that the standard of rehabilitation for the VA has been very strong. There are lots of people who have had their legs blown off who are walking because the prosthesis that the VA put together were real good. And I think the rehab effort of the VA needs to get into the current types of illnesses that are existing. How can it do that? Well, I think that number one there are anecdotal reports of successful rehab modules. I think the one that we heard an anecdote about the guy who went out to California and did the sauna, vitamin, water therapy and said he felt a lot better. That's a low risk. It's the sort of thing that a VA rehab unit all around the country could adopt if it works, and it could be delivered. It could be a good deliverable. And it would be possible to determine whether or not it is effective by having a multi-center trial, having a data coordinating center, having the guys in California, Dr. Root I think it is, I don't know, the guy who have spent 17 years doing it, try to train other people to do

it, and in 6 or 8 centers see if it works. He doesn't control the analysis, it isn't just done at his center, the VA tries to do it an see if it works at their center. So that that module of taking anecdotal reports, testing them in a multi-center environment, with a data coordinating center, with oversight by both vets and by scientists can then help the VA say at each budget cycle, "Yeah, this is a new widget. We think this is good. We should adopt it." And something that would work within the strong VA tradition of doing rehabilitation for the wounded warrior. Thank you.

Dr. Stephen Thacker, Moderator

Our last panelist is David Ozonoff. He's the chair of the Department of Environmental Health at Boston University School of Public Health, the medical director of the Boston Environmental Hazards Center. Dr. Ozonoff received his medical degree from Cornell, and his MPH degree from Johns Hopkins. Dr. Ozonoff's research is centered on epidemiologic studies of populations exposed to toxic agents, especially the development of new methods to investigate small exposed populations. Dr. Ozonoff.

David Ozonoff, MD, MPH
Chair, Department of Environmental Health
Boston University School of Public Health
Medical Director, Boston Environmental Hazards Center
Boston VA Medical Center
Boston, Massachusetts

First of all, I'd like to thank Dr. Haley for taking my pituitary adrenal axis out for some vigorous exercise. I'm going right from here for some intravenous Zantac.

My task was to discuss research strategies for Gulf War illness, and every time I sat down to think about it, my mind went completely blank. In fact, I even had an EEG done at one point that showed there were only 2 neurons firing – I was using one to breath with and with the other I was drinking coffee. However, I've come to the point where I do have to say something, so first let me lay the ground rules for what I'm going to say. This is the first overhead, "Back to Basics." And that's really, I think, the gist of what I want to talk about, which is a strange thing in a way to have as a theme for my talk because I've spent 3 decades working with community groups on very specific problems of contamination with urgent, immediately asked for solutions to their problems. However, I'm going to come out somewhere slightly different here.

Here are the assumptions that are the basis for my remarks here. First of all, I'm going to use a very literal meaning of the word "strategy." I'm going to assume that Gulf associated illness is real, and that the goal is the explanation of unexplained illness. So, let me take them one at a time.

The first one, if you look at the military meaning of the word "strategy," the word strategy incidentally comes from the Greek word for general or generalship, so it's very appropriate in this context. And here's the military definition, "Utilization in both peace and war of all of a nation's forces through large scale, long-range planning and development to insure security or victory." Whereas, "tactic" refers to winning a particular battle. So, my focus really in these brief remarks is going to be on the big, long-term picture, not the immediate, short-term one. It's not because I think the latter is unimportant or of lower priority, but because I thought my task defined it out of the scope of what my remarks were supposed to be.

I'm going to make the following assumption, which is not necessarily shared by all researchers on it, but it happens to be the one, after having worked on this for about 4 or 5 years, this is my prejudice on the matter, that Gulf illness is real and here's what I mean by that – that unexplained illness among some veterans of the Persian Gulf conflict refers to health effects from exposure to physical, chemical, or biological sources which were specific to the Gulf environment. In other words, there was something in the Gulf in exposure but for which they would not have gotten the particular illness they got at that particular time. So, what I'm essentially saying here is that if they'd gone to Bosnia, or if they'd gone to Somalia, or Kosovo, or somewhere else where there was combat, they wouldn't have gotten Gulf War illness, that this is not combat stress or anything else. This is something that was specific to the Gulf environment as yet not known exactly what it is. I'm not ruling out the possibility that stress is a co-factor or an effect modifier or confounder, but it's not in the foreground. What we need to do as far as a strategy is find out what the physical, chemical, or biological factor is which is producing this illness.

And now the third thing is, my remarks assumed that the strategy was to explain Gulf War illness. In other words, to move it from unexplained illness to explained illness. The reason I think that's an appropriate goal is because it's applicable to both prevention and to treatment, and therefore, it seems a reasonable thing to strive for.

I'm only making this explicit, it might seem like sort of an obvious thing to want, is that you could actually try and devise preventive strategies or treatment strategies that made no reference to the explanation or didn't know what the explanation was. I'm going to make a remark about that in a minute. I suspect that's not going to work if we try and do it. If we just try it by trial and error or, you know, trying one thing after another. Okay. So, what should this strategy be? What is the long-term, large-scale plan that's going to achieve victory in this very confusing conflict against a mysterious and unexplained illness? That's the hard part. I'm afraid that my answer is going to be not very satisfying. At least, it's not very satisfying to me, but if you want to know what I truly think, this is what it is.

In order to motivate it, let me actually just make a brief reference to this book that I brought. It's not exactly current. I'm a book review editor and this book reached me 114 years late. It was published in 1885. It's entitled *Asiatic Cholera*. And it was written just 7 months after the actual

cause of cholera, which is the cholera common bacillus which was discovered by Koch in Egypt. This is 7 months after the true discovery of cholera. If you actually look at the table of content of this book, it will sound like the executive reports on Gulf War illness. It just goes through all the same kinds of questions because it was at a time when that very serious epidemic disease was not completely understood. There are 2 things of special pertinence and interest about this book for purposes of this meeting. One is that there was an immediate recognition within 7 months that Koch had the answer, right? That basic research and his answer was based on a basic research technique which had nothing to do with cholera. It was the ability to culture bacteria in pure culture, that that was correct. The second thing is because I'm an epidemiologist, and the father of epidemiology is John Snow, a famous epidemiologist whose investigations of cholera in 1853 and 1854 are held up as the model for epidemiologic studies of the kind that Dr. Haley was talking about. This is a 400 page book. There are exactly 20 lines devoted to John Snow in this book. I'm just going to read one sentence from it. It says, "Snow's writings show that he was a careful observer and, although he remained in ignorance of the true nature of the infecting agent, he nevertheless should receive the credit of having ascertained the manner of its behavior and a favorite mode of its dissemination."

Now, you could read that as saying, "See, epidemiology is the way to go. In fact, here is a guy 30 years before the bacillus was discovered who had it all figured out." The point is, nobody paid any attention to John Snow. He had almost no influence at all. He only became the father of epidemiology in 1930 when Wade Hampton Frost resurrected him from obscurity and promoted him into this exalted position.

So, what does all this have to do with Gulf War illness? Here's what I think it is. First, I think that adequate explanation, which is the goal here, is likely to be a by-product of good basic research, and not solely the product of targeted research agendas. A fruitful strategy is going to require providing additional resources for basic research. Absolutely basic research, initially directed to understanding certain specific areas likely to be pertinent, but not specific to Gulf War illness. Now here, I'm thinking about things like immunotoxicology, neurotoxicology, inhalation toxicology, and so on. And I think that means that DoD, VA, CDC and NIH should be making strong, and stronger arguments for supporting basic research. And it should be supported even by people who are interested in human illness. It is important what we find out about mice and rats, how ever they market and package it for the appropriate committees in Congress, GAO panels, Presidential commissions, whatever. It's important to understand that basic research for its own sake is probably going to be the place where the answer is found, just as it was in the case of cholera, and recognized immediately.

Second, support for certain kinds of basic research that are not currently being pursued should be promoted. Here, I'm talking about some of the things that Dr. Haley was talking about, new methods brought in from other disciplines, brought into epidemiology from other disciplines, to discover patterns. Factor analysis is one of the things that epidemiologists have used. It's sort of

a magic black box, nobody knows exactly what it means, but there are other techniques used for airline scheduling, used for handwriting analysis, things that could be brought in – basic research techniques to look for the kind of patterns that might provide the proper case identification and case definition. And I must say, Dr. Haley, I disagree with you about just picking out any case definition. If you mis-classify outcomes, you run into the same problem you do when you mis-classify exposures. You don't see what you're looking for.

Third, there should be work groups within the agencies, just as there are now, whose job it is to surveil the basic science literature, this exploding new knowledge in biology, and apply it to Gulf War illness thus closing the loop.

And finally, we should continue to have a major effort in developing our exposure and outcome infrastructure. Specifically here, I'm talking about the things that Dr. Heller was talking about. CHPPM has done a really terrific job in collating this information and putting it in a form where it's usable. But a great deal more has to be done in that regard in making it more accurate and more complete. And there should be efforts devoted to medical bioinformatics so that services have a common record keeping system, and in fact, medical outcomes can be followed across services and prospectively into the future.

Whatever, the specifics of these things, I think the general idea here is to establish a 2-way link between basic research, which is going to be, I believe, the key to all this – the basic mechanistic research of what happens at the cellular and molecular biology level, and outcomes of particular interest, like MCS, neuropsychology and the immunology applications. In keeping with the definition of "strategy," of course this is a long-term project, but one that I think will bear fruit within a relatively short amount of time. Thank you.

Dr. Stephen Thacker, Moderator

As our panelists return, we're supposed to have discussion time among the panelists, but I think it's only fair to, in the last 15 minutes, allow some of the audience address questions to members of the panel.

Discussion

C. Kirt Love
Signing My Life Away
Copperas Cove, Texas

First of all, I'd like to apologize to Jack Heller for interfering or interrupting with the speech. It wasn't my intent to interrupt and I hope I didn't disrupt things badly. The key problem I'm

running into here that I wanted to address, this sample was not stored in a controlled environment. So, one of the things is that the sample may not be viable. I was wondering, what would be considered a viable sample and what is deemed a viable sample?

Dr. Jack Heller

It depends on what you're looking for. If you're looking for something like depleted uranium, heavy metals, even some long-lived organics, a sample that old could be good. If you're looking for, you know, chemical warfare agents, you'll probably never find them. If you're looking for volatile organic solvents, obviously you'll never find them. So, it can be used to look at certain long-lived things, but not the things that are very labile and very volatile.

Mr. Kirt Love

But you would be interested in this sample?

Dr. Jack Heller

Yes. The thing we really want to know is where it came from in the Gulf.

Mr. Kirt Love

I have that written. I need to re-write it. I also have a night vision block off a T-72 Tank that has never been DU tested. Would you be interested in that sample also?

Dr. Jack Heller

I don't know. Where is our health physicist? Is that stuff safe if it's sealed up? Can you transport it? I don't know.

Mr. Kirt Love

The outside of it's been wiped down, and I keep it pretty close to me.

Dr. Jack Heller

I assume it's sealed?

Mr. Kirt Love

Yes. That's what I was trying to say, that many of the people that I deal with, a lot of the

veterans have samples of this nature, but they have not stored them, like I said, in scientific controlled fashion. So, if then it's not admissible, I can save a lot of other people a lot of time trying to bring it forward.

Dr. Jack Heller

Well, the Office of the Special Assistant, when he goes around, he makes the offer to people, if they have a particular item that they'd like to get tested, he's been accepting it. Lately, I guess he's become a little more restrictive. He's more interested in ones that may have caused the person a health problem. But again, we're still willing to look at things. I mean, the one thing obviously, is it hasn't undergone a chain of custody. Somebody could say, "Well, you know, they could spike it." That's the one thing you don't know. But, you know, we're still interested because there are a lot of areas that we don't have good samples from. So, the more areas we get samples from, the better it is.

Mr. Kirt Love

Well, I was a souvenir collector, unfortunately. And that might be part of my problem could be some of the souvenirs that I brought back with me. Thank you for your time.

Richard Wadzinski
United Veterans of America
Godwin, North Carolina

Once again, I want to reiterate I did my time, retired from the Air Force in '94. Then in '97, my liver quit, failed, I went into a coma for a couple of weeks, had 2 hours to minutes to live, got a transplant. It's a day-to-day, every day's a gift. I am going to start on this end of the panel with the DU folks. I slept on the DU on the plane flying 20 hour days. I don't know what kind of effect that could have. The next man, what did you do again Mr. John.

Dr. John Feussner

The first name is easier to pronounce than the second one, isn't it. I'm the Chief Research Officer for VA, sir.

Mr. Richard Wadzinski

Okay, research. You talk about research. Research me, not mice. I'm unexplained illness, 150 percent, appeal still in. I don't sleep. I get migraines. I pass out driving the car. And I could go on, and on, and on. In Duke University, they've been taking care of me. They've found nothing to figure out what the problem is. Then, for the next gentleman about this research, due to my

condition, I can't be helped by that, this doxycycline program. So, who is going to find something to help me? And the other gentleman about birth defects, my daughter's got birth defects. The retirees, after we are out, no one gives a damn about us. We're kicked to the curb. All the research done, we've been left out of the picture. For you ma'am, don't call us a beggar. I deserve everything coming to me. However you said that, it offended me. I am not a beggar and they deserve to take care of us. Years ago when I came in, the government told us free medical and free dental for life. And you see what's going on. Now I've got to pay for supplements. I've got to pay for dental. It's not right. It needs to be taken care of. And the last statement I have, I'd like to see CDC take control of this whole mess and get the VA and DoD out of it. CDC can run the program so you don't have any biased, conflicts, whatever, and the government trying to hide stuff. That's what I'd like to see done. Thank you.

Dr. Rebecca Bascom

Could I say one thing? Which is, the analogy that I described, the warrior and the beggar, is something that I have thought of as I've been listening to people here and hearing the problems of the veterans as they are trying to get care, and they're feeling like they're having to beg for something that they thought was part of the deal when they enlisted. I think that that is a very harmful thing to do to a person who has other wounds from which they are trying to cover, and I apologize if that was . . .

Mr. Richard Wadzinski

Can I interject one more thing? I went 8 months before I received any help. Denied welfare, I had to get Senators and Congressman to get the VA off their butt to get me a check. Social Security doesn't pay until 8 months down the line. I almost lost my house and everything. I'm not a beggar. I'd like to be back to work. I was a medic out helping people and stuff. I can't do anything anymore.

Dr. Rebecca Bascom

And it's wrong for you to have to wait for resolution of things.

Meryl Nass, MD
Freeport, Maine

My expertise is in biological warfare, anthrax, and anthrax vaccines. Now what I would like to do, and I think it only fair, is to usurp about 5 minutes of your time because a considered look at the possible role of vaccines is not taking place at this meeting, and has in fact never taken place.

Dr. John Feussner

Excuse me ma'am . . .

Dr. Meryl Nass

No, excuse me . . .

Dr. John Feussner

Can I say something? I actually was intending to answer the question that the veteran asked, and I was going to wait to answer some of his questions, which I find very troubling and useful to have a dialog on. I don't want to wait until you get done making your statement to answer his question. Would it be okay if I interrupted you and answered his question?

Dr. Meryl Nass

Please.

Dr. John Feussner

Thanks. There are 3 things I want to say. I wish, believe me, as a physician I wish I knew how to make you better, because if I did I would. But, I would like to reflect on what Dr. Ozonoff said, that we should do research that improves our understanding about basic mechanisms. I thought you said that you had a liver biopsy, or a liver transplant. It's important to understand that the first liver transplant done in the United States of America was done and sponsored by VA research, and it was done by a VA surgeon, and that basic research was started 20 years ago. I believe it was Dr. Starzl. He intended for that basic research to benefit people and to benefit people through time. But it took quite a long time from that basic understanding about transplant biology to be transferred such that it could help people.

Mr. Richard Wadzinski

I wouldn't be here if not for that. I understand that.

Dr. John Feussner

You're taking the words right out of my mouth. I think that, to a degree, you're living proof that the investment in that basic research is very beneficial. The second thing I wanted to say in answer to your question is, I'm quite sensitive to what you commented about the basic research in animals and mice and that that's useful. I am not a basic researcher myself. I am a clinical researcher, that is, doing work on individual patients. Our research portfolio is balanced in that regard, that is, the basic work that's probably not going to provide us answer for years and years

and the clinical work, some of which is trying to get at some useful treatments now like the liver transplant work that was done earlier. Thank you. And I'm sorry to interrupt.

Dr. Meryl Nass

What I was saying before was that there has not yet been an American research protocol that actually looks at the effect of the vaccines that were administered at the time of the Gulf War, and that several expert panels which were convened and asked to look at this among other issues did not look at any data, but chose to assume that given what we know about vaccines, there's no reason to expect that vaccines could have contributed to the illness. These panels all stopped there. This may explain why no vaccine experts were invited to present their findings at this meeting today. Now, it so happens that the first study came out a month ago in ***The Lancet***. It's already been mentioned. That was by Unwin and Wesley. It was not an ideal study because it relied on recall of vaccination by troops in the Gulf War, and there were 2 control groups, non-deployed Gulf Era vets and Bosnia vets. There were over 4,000 in each group. The best correlation between subsequent illness was vaccination, and it was statistically significant. This has not had the impact it deserves because it's the only study to look at it, and it shows that vaccination had the greatest effect on subsequent illness, epidemiologically.

Now, it turns out, when you actually look at the, the devil is in the details as we've said before, when you actually look into this vaccine, you find that there are serious problems with its manufacture. Nobody did supplemental testing before the vaccine was administered to Gulf vets. But because of questions about the vaccinations given to Gulf vets, supplemental testing was ordered by the Secretary of Defense before the currently round of vaccinations. It so happens that after the supplemental testing was done last year, 8 lots passed and 11 lots failed. So more than half of the vaccine stockpiled that was examined was not fit for human consumption.

Dr. John Feussner

Excuse me ma'am. I came here with one specific purpose in mind, and that is, I was anxious to listen to veterans whom I work for. And I'm concerned that if you continue this way, I'm not going to get to do that. Can you say about how much long we have, and I apologize to Stephen for interrupting, but would it also be possible to give the veterans some preference at the mike and perhaps allow them to speak first and then the rest could make your statements at your leisure.

Mr. Albert Donnay

For a day and a half we've been doing it this way. At the suggestion of the Chair, I suggest we continue to do it this way. We're patiently waiting in line.

Dr. Meryl Nass

Anyway, let me have my minute. It so happens that there exist no long-term safety studies on this vaccine. All the safety studies are short-term, up to 28 days only. We were told by Dr. Heller that there is now documentation that 7000 Gulf War Veterans received anthrax vaccine. If that data is good, we have a cohort that can be studied. The problem is that most of the veterans did not have the vaccinations put in their shot records, and so we are unable to document who got the vaccine and who did not. If we use this cohort, we need to choose an appropriate control group that is not from the Gulf War because we need to be certain that they did not get the vaccine. We were also told that 150,000 doses were administered. I have some evidence that this may not be the correct number. Certainly we gave over 50,000 doses to Great Britain, and we gave doses to Canada, and to other nations. If we were in such short supply that we had only 150,000 doses left, why did we give so much overseas? I think also there are issues about how many doses people got. There is different data from unpublished DoD studies that suggest that the official DoD numbers are inaccurate. So, what I would suggest to you is that this clearly, the impact of the anthrax and the other vaccinations clearly is dying to be studied, that we do have cohorts, we also have people who work at Fort Detrick who have been receiving this vaccine on a regular basis over a long period of time. These cohorts should be studied, controls should be selected properly, and that the raw data, and exactly how the numbers are arrived at needs to be made clear so that these studies can be reviewed carefully by all concerned. If anyone is interested, I have an article on anthrax vaccines in this month's issue of *Infectious Disease Clinics of North America* on this subject.

Dr. David Ozonoff

Just a comment for information. This is not a subject that is ignored. In fact, some of the basic research supported by Dr. Feussner's office in the Boston Environmental Hazard Center is specifically directed into looking at the effects on immature immune cells, both T and B-cells from, that are activated by vaccines from exposure to components of oil well fire smoke which interacts with the AH receptor. So, in fact, it is a subject that is the focus of basic research at moment.

Dr. Stephen Thacker, Moderator

We've gone past our time, but I'll allow one more question.

Mr. Albert Donnay

My question is for Colonel Gary Gackstetter. Dr. Gackstetter, I understand that you were one of the senior people in charge of the epidemiology of the CCEP program when you worked in Dr. Joseph's office, and that you also for 4 years, from 1995 to present, were the Department of

Defense representative on the Federal Interagency Work Group on multiple chemical sensitivity whose draft report was issued for public comment last August. In that draft report, there is no mention of any Department of Defense data on multiple chemical sensitivity. And two weeks before this meeting when I called and asked you to please bring the data on multiple chemical sensitivity collected by the Department of Defense in the Comprehensive Clinical Evaluation Program-Phase III, which was used for 6 months, a questionnaire developed by General Blank with help from MCS experts, you told me that you didn't think that those data were valuable, but you also admitted that you hadn't looked at them, and that no one has looked at them. And I've confirmed that from other sources.

The DoD CCEP data were supposed to be available to independent researchers. These data are not available to independent researchers. I'd like to ask if you'd be willing to make those available and why, in the federal report, the DoD did not disclose either the data on MCS from the study it did with CDC of the Iowa active duty which was reported in *JAMA*, or the data on MCS which were reported in the *JAMA* study last September in the a Pennsylvania study done with CDC?

COL Gary Gackstetter

I think we can do this pretty quickly. I'm not aware of that. So that's easy.

Mr. Albert Donnay

You're not aware of what?

COL Gary Gackstetter

Anything that you say, in fact.

Mr. Albert Donnay

Are you denying we had this conversation? Two weeks ago?

COL Gary Gackstetter

I am aware that we spoke. I did ask some questions. I began my epidemiologic work in October of '94. I believe you're speaking of a period before I got there.

Mr. Albert Donnay

The period of the questionnaire, as we've discussed repeatedly over the years, was June to

December of '94. You told me . . .

COL Gary Gackstetter

I'm not aware of it.

Mr. Albert Donnay

And the other reports of the CDC studies in Iowa and Pennsylvania, you never heard of those? You said you never heard of it. I'm clarifying your response, Dr. Gackstetter.

COL Gary Gackstetter

Sure. Let me see if I can explain. I have no idea of the survey instrument of which you speak. So, when I inquired as to where this was, no one knew of it. Now, that could be that either it doesn't exist or they didn't know of it. As far as the information out of CDC or Iowa, I have no idea what those investigators were up to. They are completely and totally independent, so you're going to have to ask those PIs as to what they have to say. I have no control over any of that.

Mr. Albert Donnay

They were funded by DoD and you were the DoD's representative on the MCS Work Group to report on MCS research. There is a discussion in that report about MCS research by the DoD, but it does not mention these studies. So, I assume from that then, when you looked you didn't read the *JAMA* articles?

COL Gary Gackstetter

I read very carefully the *JAMA* articles.

Mr. Albert Donnay

The data in the *JAMA* article that you read show the prevalence of multiple chemical sensitivity in the deployed was 5 percent and the prevalence in the un-deployed was 2 percent, that was both current and over the last 6 months. That's an odds ratio of 2.5. Perhaps you could then explain why the Department of Defense has not diagnosed MCS in any cases. When they reported to the Institute of Medicine on these overlapping disorders, they reported 1.3 percent with fibromyalgia, 0.3 percent with chronic fatigue syndrome, and the Department of Defense did not provide any data on MCS to the Institute of Medicine to review.

Dr. Stephen Thacker, Moderator

Okay. We have run out of time, and the sessions that next start on finalization of research recommendations start in less than 10 minutes. So, in deference to those sessions, we'll have to close now. If you have specific questions to any of the investigators here, I'm sure they'll be happy to speak with you. Thank you very much.

The session adjourned.

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